THE PHARMACOKINETICS, PHARMACODYNAMICS AND PHARMACOGENETICS OF DOLUTEGRAVIR AND COBICISTAT IN THE TREATMENT OF HIV

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

Emilie Rachel Sarah ELLIOT

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This research was carried out at the St Stephen's AIDS Trust, Chelsea and Westminster Hospital, London, United Kingdom (UK) and at the Department of Molecular and Clinical Pharmacology University of Liverpool, UK.

I declare that this thesis is the result of my own work and the material contained in it has not been presented, wholly or in part, for any other degree or qualification. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Emilie Rachel Sarah Elliot

To Eva and Chantal

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ACKNOWLEDGMENTS

'The pursuit of PhD is an enduring and daring adventure' - **Lailah Gifty Akita**

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Finally, I dedicate this thesis to my mum, Chantal, who passed away when I was a child and to my daughter Eva, who was born during the second year of this PhD. I hope to make them both proud.

LIST OF PUBLICATIONS

JOURNAL ARTICLES included in thesis

Elliot ER, Neary M, Else L, Khoo S, Moyle G, Carr DF, Wang X, McClure M, Owen A, Boffito M. *ABCG2* 421C>A, *UGT1A1**28 and *NR1I2* 63396C>T Independently Influence Dolutegravir Concentrations in Plasma. Accepted in J Antimicrob Chemother subject to revisions.

Elliot ER, Bisdomini E, Penchala SD, Khoo S, Nwokolo N, Boffito M. Pharmacokinetics (PK) of ethinylestradiol/levonorgestrel co-administered with atazanavir/cobicistat. HIV Res Clin Pract. 2019 Jul 23:1-10. doi: 10.1080/25787489.2019.1638077

Elliot ER, Cerrone M, Else L, Amara A, Bisdomini E, Khoo S, Owen A, Boffito M. Pharmacokinetics of dolutegravir with and without darunavir/cobicistat in healthy volunteers. J Antimicrob Chemother. 2019 Jan 1;74(1):149-156. doi: 10.1093/jac/dky384.

Elliot ER, Wang X, Singh S, Simmons B, Vera JH, Miller RF, Fitzpatrick C, Moyle G, McClure M, Boffito M. Increased dolutegravir peak concentrations in people living with HIV aged 60 and over and analysis of sleep quality and cognition. *Clinical Infectious Diseases*. 2018 May 16. doi: 10.1093/cid/ciy426.

Elliot ER, Amara A, Pagani N, Else L, Moyle G, Schoolmeesters A, Higgs C, Khoo S, Boffito M. Once-daily atazanavir/cobicistat and darunavir/cobicistat exposure over 72 h post-dose in plasma, urine and saliva: contribution to drug pharmacokinetic knowledge. *Journal of Antimicrobial Chemotherapy*. 2017 Jul 1;72(7):2035-2041. doi: 10.1093/jac/dkx108.

Elliot E, Chirwa M, Boffito M. How recent findings on the pharmacokinetics and pharmacodynamics of integrase inhibitors can inform clinical use. *Current Opinion in Infectious Diseases*. 2017 Feb;30(1):58-73. doi: 10.1097/QCO.0000000000000327.

Elliot, E.; Mahungu, T.; Owen, A. Current progress in the pharmacogenetics of infectious disease therapy. In Genetics and Evolution of Infectious Disease,2nd ed.; Tibayrenc,M.,Ed.; Elsevier:Amsterdam, 2017. Doi 10.1016/B978-0-12-384890-1.00020-0

Penchala SD, Fawcett S, Else L, Egan D, Amara A, **Elliot E**, Challenger E, Back D, Boffito M, Khoo S. The development and application of a novel LC-MS/MS method for the measurement of Dolutegravir, Elvitegravir and Cobicistat in human plasma. *Journal of Chromatography B. Analytical Technologies in the Biomedical and Life Sciences*. 2016 Aug 1;1027:174-80. doi: 10.1016/j.jchromb.2016.05.040. Epub 2016 May 27.

Elliot E, Amara A, Jackson A, Moyle G, Else L, Khoo S, Back D, Owen A, Boffito M. Dolutegravir and elvitegravir plasma concentrations following cessation of drug intake. *Journal of Antimicrobial Chemotherapy*. 2016 Apr;71(4):1031-6. doi: 10.1093/jac/dkv425. Epub 2015 Dec 17.

JOURNAL ARTICLES non-included in thesis

Elliot ER, Theodoraki A, Jain LR, Marshall NJ, Boffito M, Baldeweg SE, Waters LJ. Iatrogenic Cushing's syndrome due to drug interaction between glucocorticoids and the ritonavir or cobicistat containing HIV therapies. Clinical Medicine (Lond). 2016 Oct;16(5):412-418. PMID: 27697800

Elliot ER, Singh S, Tyebally S, Gedela K, Nelson M. Recreational drug use and chemsex among HIV-infected in-patients: a unique screening opportunity. *HIV Medicine*. 2017 Jan 24. doi: 10.1111/hiv.12487. PMID: 28117545

Elliot E, Rossi M, McCormack S, McOwan A. Identifying undiagnosed HIV in men who have sex with men (MSM) by offering HIV home sampling via online gay social media: a service evaluation. *Sexually Transmitted Infections*. 2016 Sep;92(6):470-3. doi: 10.1136/sextrans-2015-052090. Epub 2016 May 24.

CONFERENCE ABSTRACTS

Elliot E, Wang X, Simmons B, Jenkin A, Pagani N, Wong S, Vera-Rojas J, Miller R, McClure M and Boffito M. Relationship between dolutegravir plasma exposure, quality of sleep and its functional outcome in patients living with HIV over the age of 60 yrs [Abstract #O_08]. *18th International Workshop on Clinical Pharmacology of Antiviral Therapy*, Chicago, USA. June 14-16, 2017.

Elliot E, Amara A, Jackson A, Moyle G, Else L, Khoo S, Back D, Owen A and Boffito M. Pharmacokinetics of once-daily dolutegravir and elvitegravir/cobicistat following drug cessation [Abstract #O_13]. *16th International Workshop on Clinical Pharmacology of HIV & Hepatitis Therapy*. W Washington Hotel, Washington DC, USA; May 26-28, 2015.

Mallik I, **Elliot ER**, Jagjit Singh G, Boffito M and Jones R. Ritonavir/ cobicistat interaction with glucocorticoids: are simple preventative measures effective? [Abstract #P21] *Fourth Joint Conference of BHIVA with BASHH*. International Conference Centre, Edinburgh, UK; April17-20, 2018.

Elliot ER, Neary N, Else L, Khoo S, Moyle G, Carr DF, Wang X, McClure M, Owen A, Boffito M. *ABCG2* rs2231142 and *NR1I2* rs2472677 Influence Dolutegravir Concentrations in Plasma [Abstract #P467]. *25th Conference on Retroviruses and Opportunistic.* Hynes Convention Centre, Boston, MA, USA; March 3-6, 2018.

Elliot ER, Cerrone M, Else L, Amara A, Bisdomini E, Khoo S, Boffito M. Pharmacokinetics of dolutegravir with and without darunavir/cobicistat in healthy volunteers [Abstract #P468]. *25th Conference on Retroviruses and Opportunistic.* Hynes Convention Centre, Boston, MA, USA; March 3-6, 2018.

Elliot ER, Bisdomini E, Else L, Penchala SD, Khoo S, Moyle G, Nwokolo N, Boffito M. Pharmacokinetics (PK) of ethinylestradiol/levonorgestrel with atazanavir/cobicistat. [Abstract #P469]. *25th Conference on Retroviruses and Opportunistic.* Hynes Convention Centre, Boston, MA, USA; March 3-6, 2018.

Elliot ER, Wang X, Pagani N, Jenkin A, Vera-Rojas J, Day-Weber I, Moyle G, McClure M, Boffito M. Steady-state Pharmacokinetics of Dolutegravir In People Living With HIV Over the Age of 60 Years [Abstract #P432]. *24th Conference on Retroviruses and Opportunistic Infections (CROI).* Washington State Convention Centre, Seattle, USA; February 13-16, 2017.

Dickinson L, Bracchi M, **Elliot E**, Else L, Khoo S, Back D, Nelson M and Boffito M. Population pharmacokinetics (PK) of dolutegravir (DTG) alone and following treatment switch [Abstract #094]. *International Congress of Drug Therapy in HIV Infection*. Scottish Exhibition and Conference Centre, Glasgow, UK; October 23-26, 2016.

Elliot ER, Amara A, Pagani N, Else L, Moyle G, Schoolmeesters A, Higgs C, Khoo S, Boffito M. Steady-state pharmacokinetics (PK) of atazanavir/cobicistat and darunavir/cobicistat once daily over 72 hours in healthy volunteers: the importance of PK forgiveness in clinical practice [Abstract #P307]. *International Congress of Drug Therapy in HIV Infection*. Scottish Exhibition and Conference Centre, Glasgow, UK; October 23-26, 2016.

Dickinson L, Pertinez H, **Elliot E**, Khoo S, Back D, Boffito M. A Semimechanistic Enzyme-Turnover Model for Simulating Darunavir/Cobicistat Pharmacokinetics over 72h Following Drug Cessation in Healthy Volunteers [Abstract #P065]. *17th International Workshop on Clinical Pharmacology of HIV & Hepatitis Therapy.* Washington Plaza Hotel, Washington DC, USA; June 8-10, 2016.

Singh S, **Elliot E**, Pasvol T, Barbour A, Platt M, Reeves I, Sathia L, Sabin C, Taylor C, Peck J and Sullivan AK on behalf of the London HIV Combined Audit & Drugs Subgroup. High rates of Sexually Transmitted Infections (STIs) and Recreational Drug Use (RDU) amongst HIV positive Men Who Have Sex with Men (MSM) attending outpatient services across London (UK) [Abstract #P065]. *15th European AIDS Conference*. Centre de Convencions Internacional de Barcelona, Barcelona, Spain; October 21-24; 2015.

LIST OF ABBREVIATIONS

µg	Microgram(s)
µL	Microlitres(s)
3TC	Lamivudine
95% CI	95% Confidence Interval
ABC	Abacavir
ABCB	ATP-binding cassette transporters sub-family B
ABCC	ATP-binding cassette transporters sub-family C
ACTG	AIDS Clinical Trials Group
ADME	Absorption, distribution, metabolism, and elimination
ADR	Adverse Drug Reaction
AE	Adverse Event
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
APO	Apolipoprotein
APV	Amprenavir
ART	Antiretroviral Therapy
ARV	Antiretroviral
ATP	Adenosine Triphosphate
ATV	Atazanavir
AUC	Area Under the Curve
AZT	Zidovudine
β	Regression coefficient
BCRP	Breast Cancer Resistant Protein
BQL	Below quantification limit
CAR	Constitutive androstane receptor
CDC	Centers for Disease Control and Prevention
CL	Clearance
Cmax	Maximum drug concentration
Cmin	Minimum drug concentration
CNS	Central Nervous System
COBI	Cobicistat
CSF	Cerebrospinal fluid

CV	Coefficient Variation
CYP	Cytochrome P450
DLV	Delavirdine
DVR	Doravirine
DNA	Deoxyribonucleic acid
DRV	Darunavir
DTG	Dolutegravir
EFV	Efavirenz
EMA	European Medicines Agency
ETR	Etravirine
EVG	Elvitegravir
FDA	U.S Food and Drugs Administration
FI	Fusion inhibitor
FTC	Emtracitabine
h	hours
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
$IC_{50/90}$	Concentration required to produce 50/90% inhibition
IDV	Indinavir
IQ	Inhibitory quotient
InSTI	Integrase strand transfer inhibitor
IQR	Interquartile range
Ka	Absorption rate constant
Kg	Kilogram(s)
L	Litre(s)
LC-MS/MS	Liquid chromatography tandem mass spectrometry
Log10	Logarithm to the base 10
LPV	Lopinavir
MEC	Minimum effective concentration
mg	Milligram(s)
min	Minute(s)
mL	Millilitre(s)
Mm	Millimetre(s)
mRNA	Messenger RNA

MS	Mass spectroscopy
MTCT	Mother-to-child transmission
MVC	Maraviroc
NFV	Nelfinavir
Ng	Nanogram(s)
MCR4	Melacortin Receptor 4
mM	Millimolar
mtDNA	Mitochondrial DNA
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NP-AEs	Neuropsychiatric Adverse Events
NR1/2	Nuclear receptor subfamily 1 group I member 2
NR1/3	Nuclear receptor subfamily 1 group I member 3
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OAT	Organic anion transporter
OATP	Organic anion-transporting polypeptide
OCT	Organic cation transporter
PCR	Polymerase chain reaction
PEP	Post exposure prophylaxis
PMCT	Prevention of mother-to-child transmission
PrEP	Cytochrome P450 oxidoreductase
PXR	Pregnane X receptor
RAL	Raltegravir
RNA	Ribonucleic acid
RPV	Rilpivirine
RTV	Ritonavir
S	Second(s)
SD	Standard Deviation
SNP	Single nucleotide polymorphism
SQV	Saquinavir
TB	Tuberculosis
TAF	Tenofovir Alafenamide Fumarate
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir
TPV	Tipranavir

UGT	Uridine diphosphate glucuronosyltransferase
UK	United Kingdom
USA	United States of America
WHO	World Health Organisation

GENERAL ABSTRACT

Dolutegravir (DTG), the second-generation Integrase Inhibitor (InSTI) and Cobicistat (COBI), the new pharmacological booster, were approved for the treatment of HIV in 2013-14. DTG changed the landscape of HIV therapy, raising standards for efficacy, safety and genetic barrier. It is now recommended as first line therapy in most major including universal guidelines and is the anchor drug for a number of new simplified ARV strategies. Meanwhile, COBI offers the opportunity to reduce pill burden in patients who require a boosted protease inhibitor, thanks to its co-formulation with atazanavir (ATV) and darunavir (DRV). It also has a lesser drug interaction profile than ritonavir (RTV), secondary to a lack of enzyme induction. Licensing data is often limited to highly selected study participants under strict trial conditions. The objectives of this thesis are therefore to address gaps in knowledge on the pharmacological behaviour of DTG and COBI in important real-life patient groups and clinical scenarios, including older people living with HIV (PLWH), women taking contraception, poorly adherent patients, DTG/DRV/COBI dual therapy candidates and genetically distinct populations. Five intensive pharmacokinetic (PK) studies were carried out, recruiting healthy volunteers and PLWH from four UK-based centers. Pharmacogenetic sampling from each DTG study was used in a final study to explore the impact of genetic variability in drug disposition genes on the PK of DTG.

The intensive PK of DTG was described for the first time in PLWH aged 60 years and over, showing a significantly higher DTG C_{max} (25%) versus younger subjects (median age 36yrs). Discontinuation rate secondary to neuro-psychiatric adverse events was 4.6% and seemed to relate to elevated drug concentrations, but the C_{max} increase was not associated with measured sleep or cognitive changes over six months in those who did continue the drug. The PK forgiveness of DTG and COBI-boosted elvitegravir (EVG), ATV and DRV was then characterised in healthy volunteers, showing a 72-hour therapeutic PK tail for DTG, 36hrs for EVG, 30hrs for ATV and 24hrs for DRV when boosted with COBI. The PK impacts of DTG co-administration with DRV/COBI and of ethinylestrodiol/levonogestrel (EE/LNG) with ATV/COBI were also investigated. Findings showed minimal changes in DTG/DRV/COBI concentrations when administered together and a 25% decrease in EE C_{24} with no significant changes in LNG when EE/LNG was co-administered with ATV/COBI. Finally, a pharmacogenetic association between DTG PK and variants in the *ABCG2*, *UGT1A1* and *NRI1/2* genes was demonstrated, particularly when combined.

The data presented in this thesis provides clinicians with key information on the pharmacology and safety of DTG and COBI in important patient groups and clinical scenarios. The significance and clinical validity of the data is discussed and an argument is made to support future research in DTG dose optimisation.

CHAPTER 1

GENERAL INTRODUCTION

CITATIONS

Elliot E, Chirwa M, Boffito M. How recent findings on the pharmacokinetics and pharmacodynamics of integrase inhibitors can inform clinical use. *Current Opinion in Inf Dis*. 2017 Feb;30(1):58-73.

Elliot, E.; Mahungu, T.; Owen, A. Current progress in the pharmacogenetics of infectious disease therapy. In Genetics and Evolution of Infectious Disease,2nd ed.; Tibayrenc,M.,Ed.; Elsevier:Amsterdam, 20

CONTENT

1.1. HIV AND ITS PHARMACOLOGY

By the end of 2016, approximately 36.7 million people were living with HIV/AIDS worldwide. As of July 2017, 20.9 million were accessing antiretroviral therapy (ART) globally.[1] This is a formidable accomplishment for a disease that was uniformly fatal three decades ago.[2] The critical contributor to this achievement has been the remarkably fast moving field of HIV pharmacotherapy.[2, 3] Early and sustained antiretroviral (ARV) use now suppresses the virus and enables people living with HIV (PLWH) to enjoy long and healthy lives with negligible risks of sexual or perinatal HIV transmission.[4-6] In the wake of these advancements, UNAIDS set the now well-recognized 2020 fast track targets: 90-90-90, aiming for 90% of PLWH knowing their status, 90% accessing ART and 90% achieving viral suppression worlwide.[7] This equates to almost 30 million people on ARV treatment and highlights the importance of meticulously characterising the pharmacokinetic (PK), pharmacodynamic (PD) and pharmacogenetic properties of the drugs involved in this ARV scale up.

This chapter provides a brief overview of the HIV viral structure, replication cycle and drug targets followed by a detailed review of the pharmacology and pharmacogenetics of the two significant agents approved in 2013-14 for the treatment of HIV, dolutegravir (DTG) and cobicistat (COBI). Gaps in the literature addressed by the research presented in this thesis are highlighted and the chapter ends with the research objectives and structure of the thesis.

1.1.1. An unprecedented epidemic

Whilst 1981 saw the first cases of the HIV/AIDS pandemic reported in the US,[8-11] the first verified report of AIDS dates back to 1959[12] and was retrospectively diagnosed

in blood samples collected from a man living in Kinshasa, now the Democratic Republic of Congo, by researchers studying the links between glucose-6-phosphatase deficiency, malaria and sickle cell disease.[12-15] It is widely accepted that HIV-1 and HIV-2 are the result of zoonotic transfers of viruses from infected primates in central and equatorial Africa,[15-17] and bush meat hunting, urbanisation and mass vaccination programs have all being implicated in the subsequent pathogenic evolution and propagation of the virus.[15, 17-20] Phylogenetic and evolutionary studies of HIV-1 place the first cross-species transmission of chimpanzee Simian Immunodeficiency Virus (SIV_{cpz}) into humans in the early 20th century[14-17] and evidence supports the hypothesis that SIV must have crossed over into humans multiple times with limited virulence and capacity for spread across humans prior to the start of the current pandemic.[14-17] In spite of this, it wasn't until 1987, that the human disease saw its first therapeutic agent approved.[21-23] Today, over 30 individual agents are approved by the Food and Drugs Administration (FDA), including more than 10 co-formulated combinations.

1.1.2. Viral structure, replication cycle and drug targets

HIV viruses are lentiviruses, a family of retroviruses for which humans and non-human primates are the only hosts.[2] HIV-1 was first isolated and described in 1983 and HIV-2 in 1986.[19, 24, 25] The hallmarks of retrovirus infection are reverse transcription and integration,[26] both of which are distinct and established therapeutic targets. HIV-1 is not just one virus, it comprises four distinct lineages, termed groups M, N, O, and P, each of which results from an independent cross-species transmission event. Group M (group major) is responsible for more than 90% of all HIV/AIDS cases and has ~11 suspected or confirmed clades (A-K), resulting from differing

evolutionary pressures and adaptations.[17, 27, 28] Clades A, B and C form the bulk of the epidemic.[19]

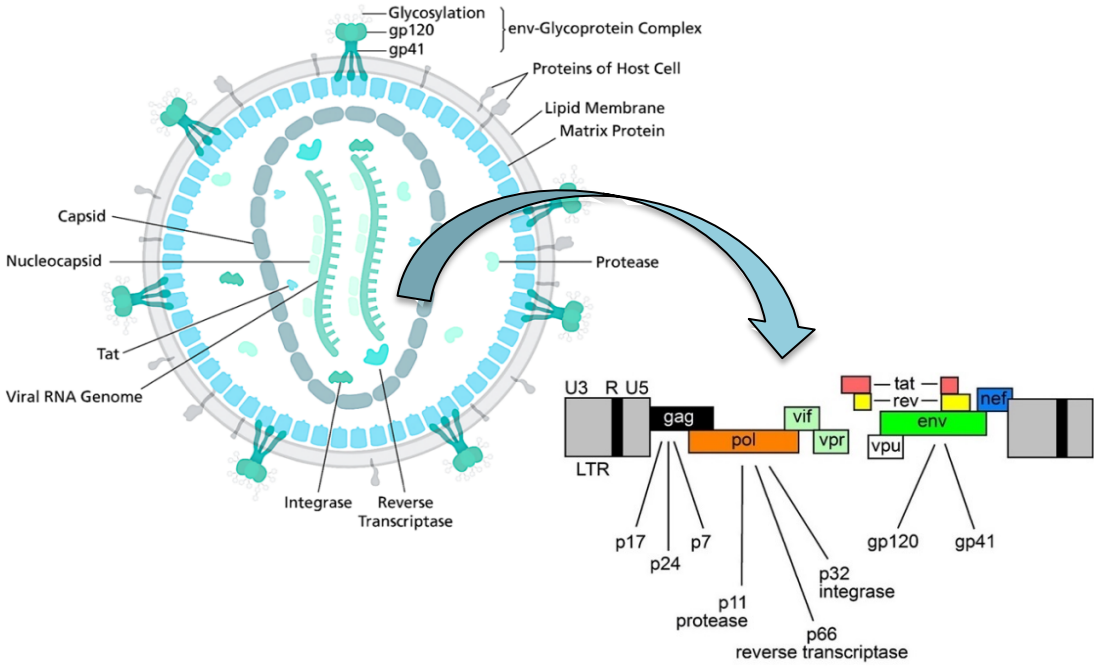


Figure 1.1: HIV-1 genomic and structural organisation (adapted and with permission from Thomas Splettstoesser [www.scistyle.com] and Hoffman *et al*. HIV book, 2011).

Figure 1.1 depicts the HIV-1 viral structure and genome organisation. Each individual virion consists of two copies of 9300 base pair-ribonucleic acid (RNA) genome, contained in a conical nucleocapsid core, itself surrounded by a lipid bilayer envelope (derived from host plasma membrane).[2, 26, 29, 30] Overall, the HIV genome contains nine genes that encode fifteen viral proteins, that are either structural (found in all retroviruses) or nonstructural ("accessory", unique to HIV).[31] The HIV life cycle and the individual steps targeted by the ARV agents currently approved or in development are illustrated in **figure 1.2**. The HIV-1 life cycle presents many potential opportunities for therapeutic intervention and some would argue that only a few have been exploited to date,[32] albeit successfully. However, still, ARVs prevent infection of susceptible cells but do not eradicate the virus in cells that already harbour the integrated proviral DNA.[2] The establishment of proviral DNA inside the host genome means that the viral

genome is replicated whenever cellular division takes place and will persist in the host even in the absence of active viral replication, which is known as the HIV reservoir.[33]

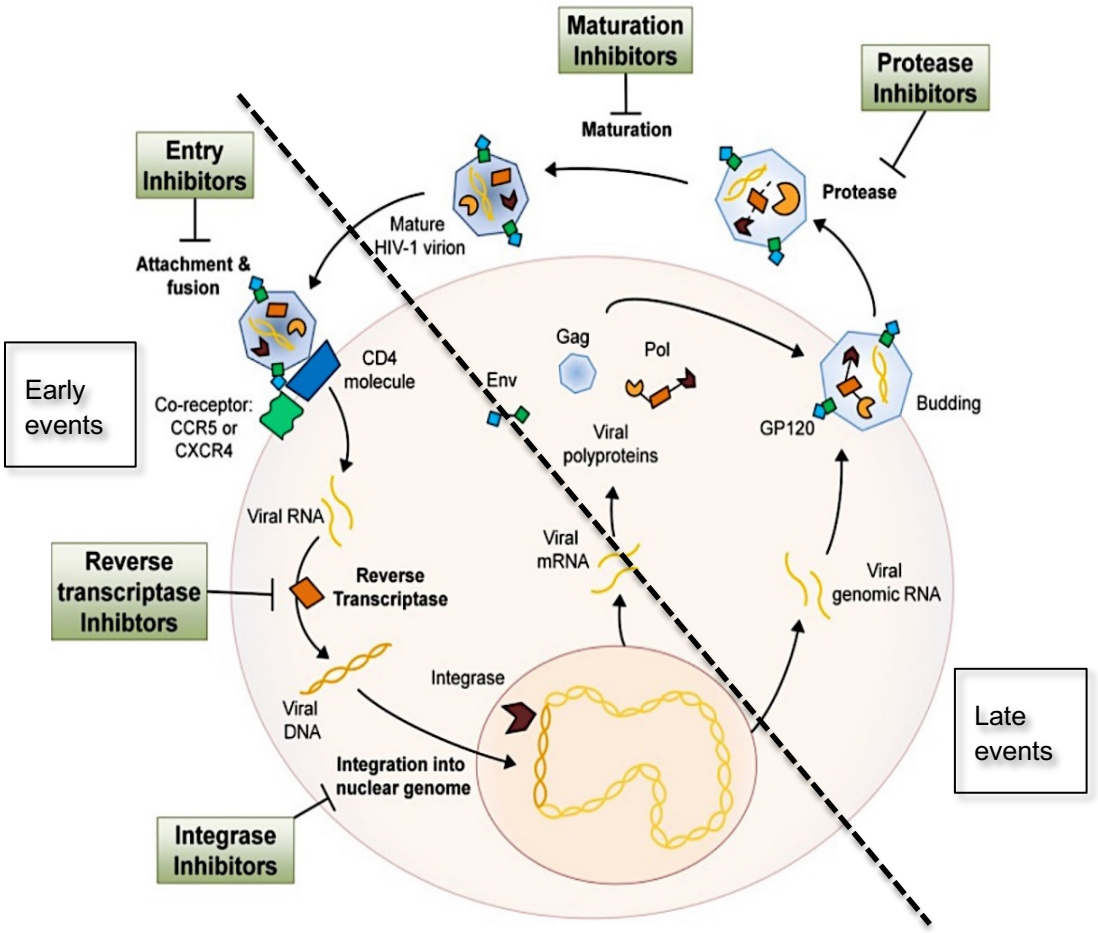


Figure 1.2: HIV life cycle in a human cell, timing of early events post-cell infection and current drug targets (adapted from Prof Stanley Bruhl, University of Amsterdam)

Triple therapy has been the cornerstone of HIV treatment since 1996. The currently used antiretroviral drugs are:[34]

i. 6 nucleoside reverse transcriptase inhibitors (NRTIs): abacavir (ABC), zidovudine (ZDV), the cytosine analogues lamivudine (3TC) and emtricitabine (FTC), and the tenofovir prodrugs tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF)

ii. 4 nonnucleoside reverse transcriptase inhibitors (NNRTIs): efavirenz (EFV), rilpivirine (RPV), etravirine (ETR) and doravirine (DOR)
iii. 3 protease inhibitors (PIs) pharmacologically boosted by ritonavir (RTV) or cobicistat (COBI): lopinavir (LPV), atazanavir (ATV) and darunavir (DRV)
iv. 4 integrase strand transfer inhibitors (InSTIs): raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG) and bictegravir (BIC)

A full timeline of FDA approvals of ARV drugs for human use is described in **table 1.1**. Trade names can be found in **appendix 1.**

1.2. A NEW ERA: THE INTEGRASE INHIBITORS

1.2.1. The HIV integrase structure

The HIV-1 integrase enzyme (IN) belongs to the retrotransposon family of proteins and is present in the initial infectious virion (40-100 copies). In vitro, it is the only viral or host enzyme necessary and sufficient to promote insertion of a donor DNA into a heterologous target DNA, at any phosphodiester bond.[35-37] The prototypical HIV-1 IN is a 288-amino-acid protein and is divided into three major domains (**figure 1.3**): an N-terminal domain, a catalytic core domain and a C-terminal domain:[35-39]

1. The Amino (N)-terminal domain, sometimes referred to as a "zinc finger", is composed of the conserved HHCC, His and Cys residues; a motif that serves to bind zinc.[40, 41] The function of the N-terminal domain is not completely clear, but it is thought to involve IN multimerisation (into dimers and tetramers) and viral DNA binding.

1980-1984	**1981** First AIDS reports in United States			
1985-1989	**1987** Zidovudine (AZT; GSK)			
1990-1994	**1991** Didanosine (ddI; BMS) Saquinavir (SQV; HLR)	**1992** Zalcitabine (ddC; HLR)	**1994** Stavudine (d4T; BMS)	
1995-1999	**1995** Lamivudine (3TC; GSK)	**1996** Indinavir (IDV; Merk) Nevirapine (NVP; BI) Ritonavir (RTV; Abbott)	**1997** Combivir (AZT/3TC; GSK) Delavirdine (DLV; Pfizer) Nelfinavir (NFV; Agouron)	**1998/1999** Abacavir (ABC; GSK) Efavirenz (EFV; BMS) Amprenavir (APV; GSK)
2000-2004	**2000** Lopinavir/r (LPV/r; GSK) Trizivir (AZT/ABC/3TC;GSK)	**2001** Tenofovir DF (Gilead)	**2003** Atazanavir (ATV; BMS) Emtracitabine (FTC; Gilead) Enfuvirtide (T-2z; HLR/Trim) Fosamprenavir	**2004** Kivexa (ABC/3TC; GSK) Truvada (TDF/FTC; Gilead)
2005-2009	**2005** Tipranavir (TPV; BI)	**2006** Atripla (TDF/FTC/EFV; Gilead) Darunavir (DRV; Tibotec)	**2007** Maraviroc (MVC; Pfizer) Raltegravir (RAL; BMS)	**2008** Etravirine (ETV; Tibotec)
2010-2014	**2011** Eviplera (TDF/FTC/RPV; Gilead) Rilpivirine (RPV; Tibotec)	**2012** Stribild (TDF/FTC/EVG)	**2013** Dolutegravir (DTG; GSK)	**2014** Cobicistat (COBI; Gilead) Elvitegravir (EVG; Gilead) Triumeq (ABC/3TC/DTG; GSK)
2015-2018	**2015** Evotaz (ATV/COBI; BMS) Genoya (TAF/FTC/EVG/COBI; Gilead) Rezolsta (DRV/COBI; Janssen)	**2016** Descovy (TAF/FTC; Gilead) Odefsey (TAF/FTC/RPV; Gilead)	**2017** Juluca (RPV/DTG; GSK)	**2018** Biktarvy (TAF/FTC/BIC; Gilead) Cimduo (TDF/3TC; Mylan) Symfi (TDF/3TC/EFV; Mylan) Ibalizumab (IBA; Thera)

Table 1.1: FDA Antiretroviral drugs approval timeline. Abbreviations: GSK: GlaxoSmithKline, Gilead: Gilead Sciences, BMS: Bristol Myers Squibb, Merck: Merck Sharp & Dohme, HLA: Hoffman La Roche, Trim: Trimeris, Thera: Theratechnologies Inc., Abbott: Abbott Laboratories, Agouron: Agouron Pharmaceuticals.

2. The catalytic core domain contains the D,D-35-E triad motif, which constitutes the catalytic active site essential to coordinate a pair of Mg^{2+} ions (or divalent metal cations) and carry out the IN enzymatic function. This motif is typical of polynucleotidyl transferases.[36] The core domain also contains key residues involved in target and viral DNA binding. Recognition of the target site is thought to be controlled by the core domain.[37, 42]

3. Finally, the C-terminal domain is mostly involved in IN multimerisation and DNA binding.[43, 44]

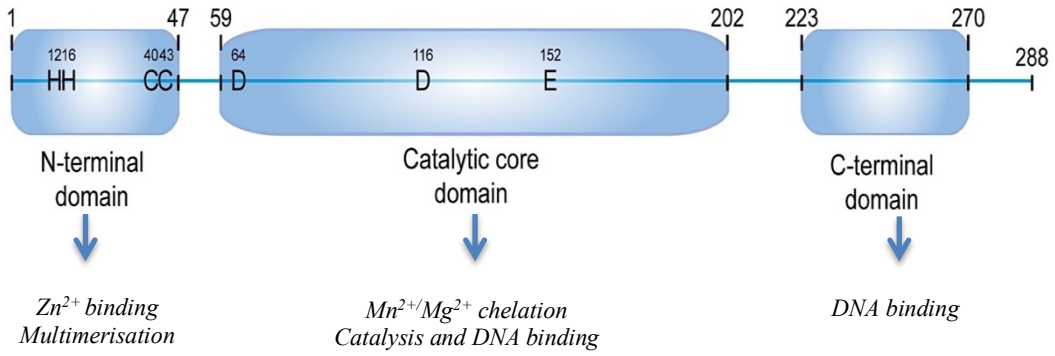


Figure 1.3: Domain organisation of the human immunodeficiency virus type 1 (HIV-1) integrase enzyme, common to all retroviral integrases (adapted from Ciuffi *et al.* 2016).[37]

The IN enzyme orchestrates several sequence-specific events required for successful stable integration, which occurs in 3 major phases:

1. Assembly with the viral DNA, endonucleolytic processing of the 3' ends of the viral DNA and nuclear translocation
2. Strand transfer or joining of the viral and cellular DNA
3. DNA repair by the host DNA repair machinery

Figures 1.4 and 1.5 illustrate nuclear translocation and HIV DNA integration sequences.

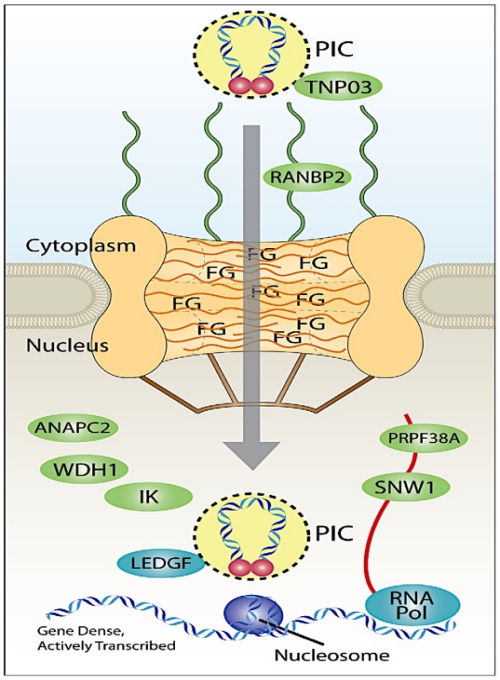


Figure 1. 4: Model of nuclear import and integration coupling. Interaction with Transportin-3 and RanBP2 shuttles the Pre-Integration Complex (PIC) through the nuclear pore and towards gene dense regions favored for HIV integration. The Ledgf/p75, co-factor then targets integration to active transcription sites. Abbreviations: F G, phenylalanine-glycine repeat sequences of nuclear pore proteins. Adapted from Ocwieja *et al.*2011

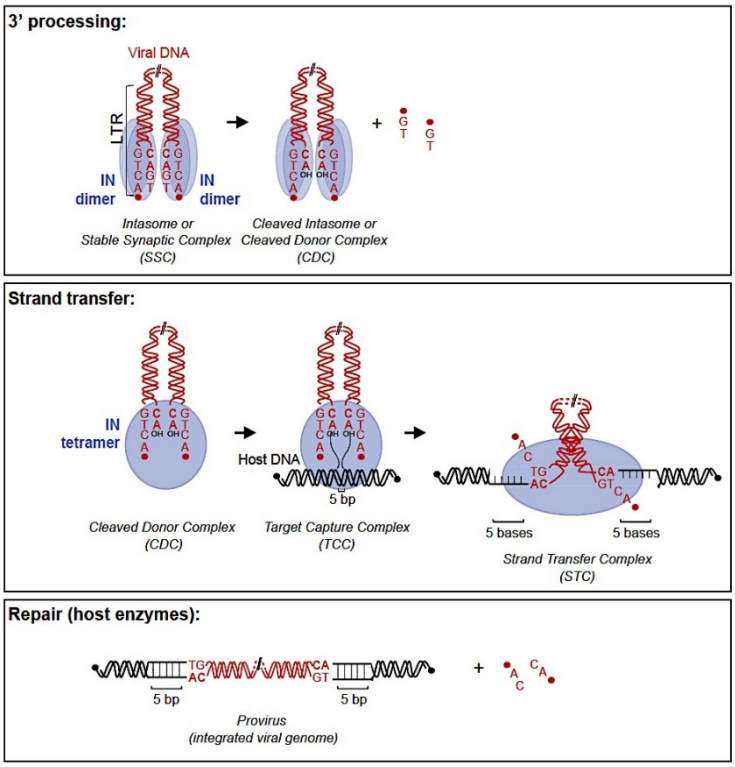


Figure 1.5: HIV DNA integration sequence (from Ciuffi *et al*. 2016)

1.2.2. The Integrase Inhibitors (InSTIs)

The integrase gene and enzyme of HIV were recognized as a potential therapeutic target susceptible to inhibition by oligonucleotides and synthetic peptides as early as 1995.[45, 46] However, the seminal study describing the first promising small compound targeting the integrase was published in 2000.[47] Structures of intasome/drug complexes (from prototype foamy virus and more recently the HIV virus itself) have revealed that InSTIs bind to the catalytic core domain of the integrase and compete with host DNA binding (**figure 1.6**).[47-49]

InSTIs are the most recently introduced ARVs. There are three established InSTIs licensed for HIV treatment naïve and experienced patients: raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG).[50] A fourth, bictegravir (BIC), was very recently (spring 2018) approved by the FDA for use in treatment-naive patients or patients with HIV-1 RNA <50 copies/mL for ≥3 months, no history of treatment failure, and no resistance to regimen components.[51] InSTIs have changed the clinical landscape of HIV therapy significantly. Data from large clinical trials have showed that, as a drug family, they are equivalent or superior to existing treatments in efficacy, display favorable pharmacokinetics and show greater safety and tolerability than PIs and NNRTIs.[52] They also benefit from paucity of drug-drug interactions (DDI), no cross-resistance to other drug classes, rapid HIV RNA reduction, action against HIV-2 and availability in single tablet regimens (STR). In a 2013 comprehensive review, Messiaen *et al.* constructed forest plots of the modified intention to treat (mITT) analyses in 16 trials illustrating the integrase inhibitors' superiority over other agents very clearly (**figure 1.7 A-C**) (data from the DTG **VIKING 3&4** studies and the BIC

licensing studies are absent). The authors concluded that in first-line therapy, InSTIs are superior to other regimens and, additionally, that InSTI use after virological failure is supported but with caution when replacing a high genetic barrier drug in treatment-experienced patients switching from successful treatment.[52]

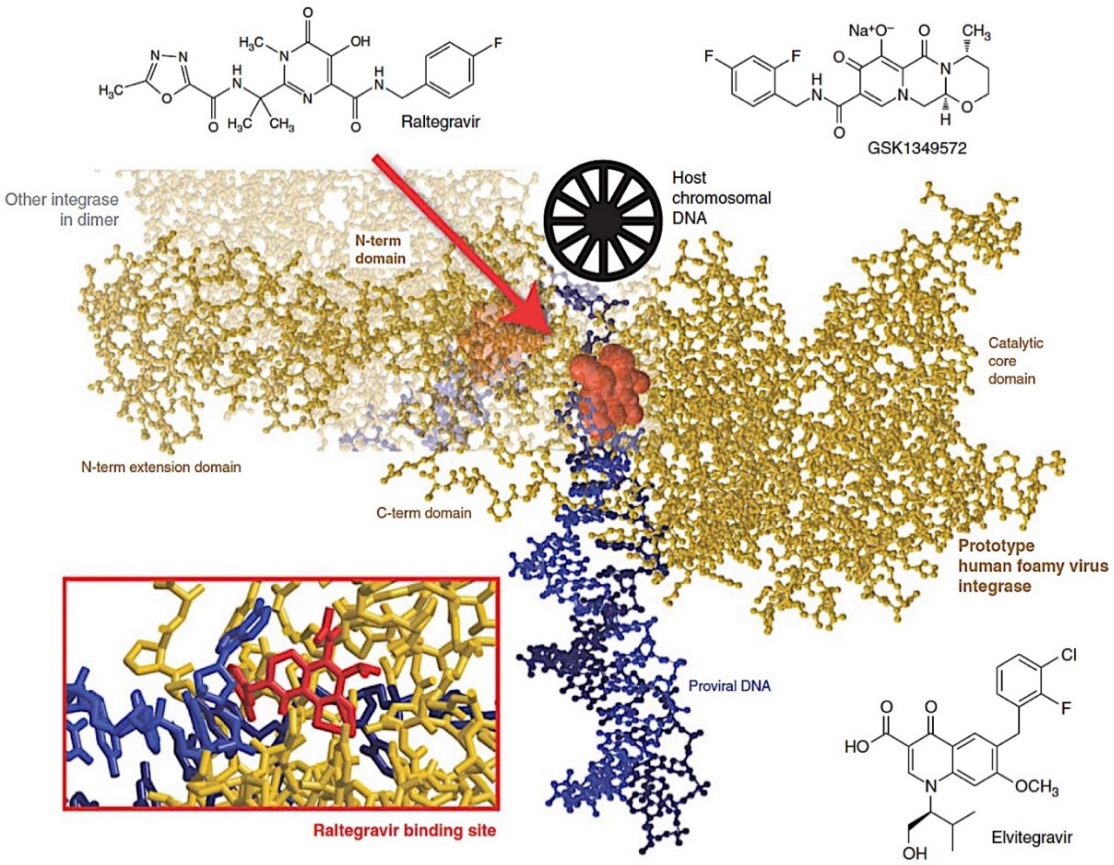
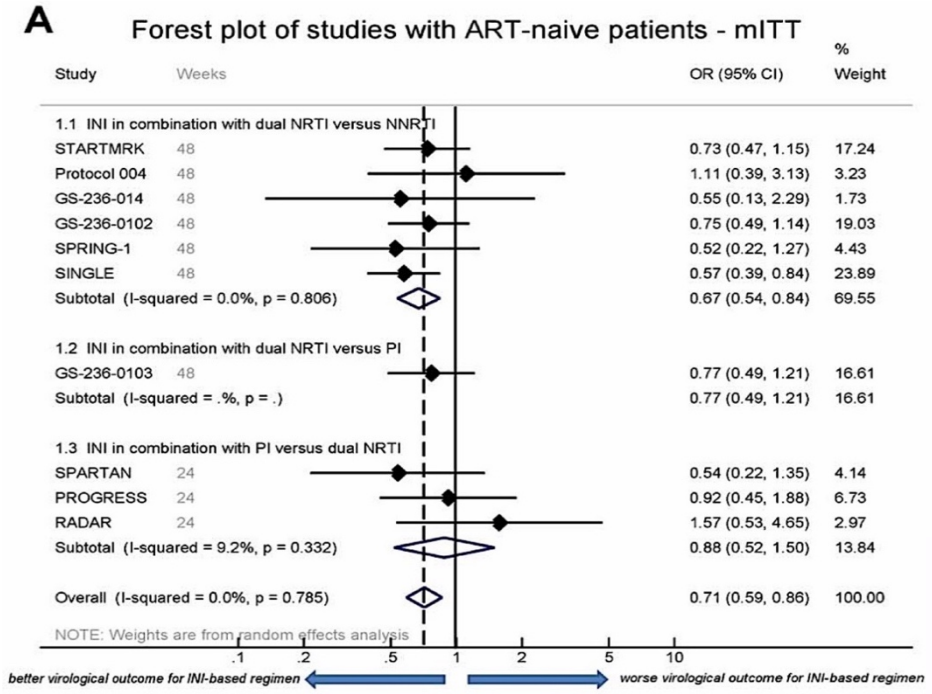
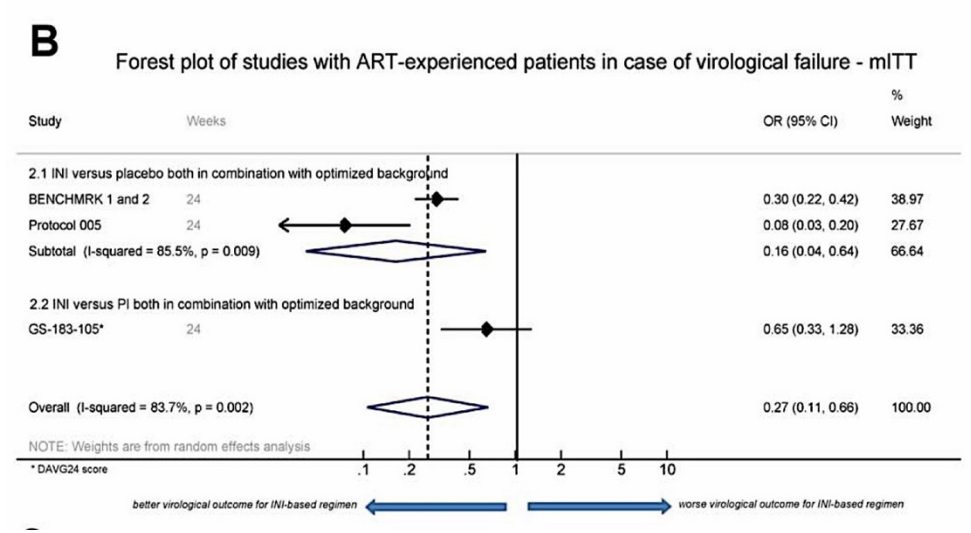


Figure 1.6: Integrase strand transfer inhibitors and the crystal structure of prototype human foamy virus integrase, complexed to dsDNA and raltegravir (from Hare *et al*. 2010, with permission). Abbreviations: N-term: amino-terminal; C-term: carboxy-terminal; GSK1349572: dolutegravir.





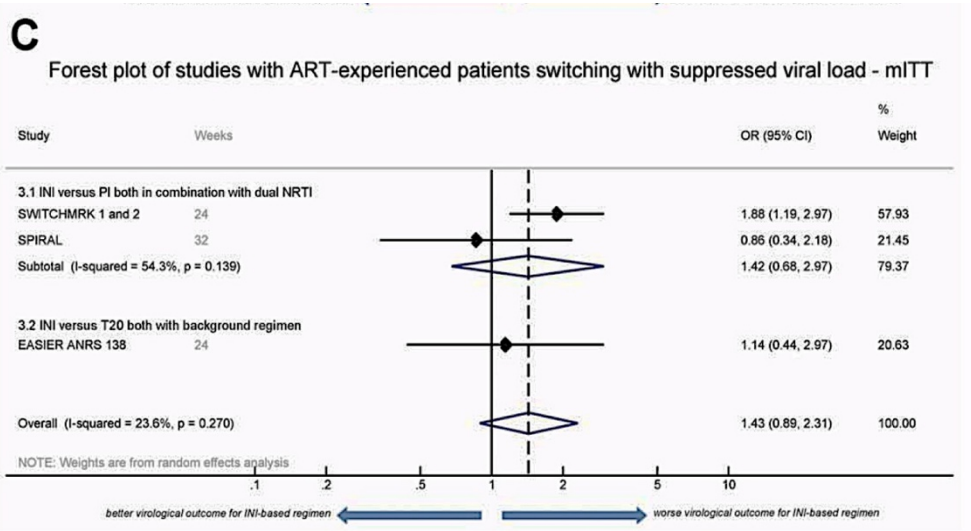


Figure 1.7: Forest plot showing the meta-analysis of mITT data extracted from studies in patients who are A)therapy naive patient B) ART experienced in case of virological failure and C) ART experienced switching with suppressed viral loads. The black line indicates OR = 1, signifying no benefit of the INI arm compared to the non-INI arm. The dotted line shows the odds ratio of all included studies. The individual odds ratios as well as the proportionate weight in the overall analysis are shown in the right column. mITT = modified intention-to-treat; ART = antiretroviral treatment; INI = integrase inhibitor; (N)NRTI = (non-)nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; OR = odds ratio

InSTIs now monopolise (DHHS/IAS-USA) or dominate (EACS/BHIVA)[53-56] first-line treatment in guidelines (**table 1.2**). They have even been credited for a marked increase seen in viral suppression and maintenance of undetectability overall.[57] **Table 1.2** shows the recommendations for first line therapy in major 2018 HIV treatment guidelines.

DHHS	IAS-USA	EACS
BIC/TAF/FTC	**DTG/ABC/3TC**	**DTG/ABC/3TC**
DTG/ABC/3TC	DTG + TAF/FTC	DTG + (TAF or TDF)/FTC
DTG + (TAF or TDF)/FTC	**EVG/COBI/TAF/FTC**	**EVG/COBI/(TAF or TDF)/FTC**
EVG/COBI/(TAF or TDF)/FTC	RAL + TAF/FTC	RAL + (TAF or TDF)/FTC
RAL + (TAF or TDF)/FTC		**RPV + (TAF or TDF)/FTC**
		DRV/ (RTV or COBI) + (TAF or TDF)/FTC

Table 1.2: North American and European 2018 guidelines for first line therapy in treatment-naïve HIV infected individuals. Abbreviations: DHHS: Department of Health and Human Service; IAS-USA: International Aids Society USA; EACS: European AIDS Clinical Society. In bold: single tablet regimen.

Whilst the InSTI compounds share the same mode of action, they exhibit different PK, PD and DDI profiles. PK characteristics for each agent are tabulated in **table 1.3**.

The second generation InSTI, DTG, is discussed below including licensing data, emerging real life experience, novel strategies and use in special populations.[58] COBI is then discussed in the context of EVG licensing, the other second generation InSTI and in the context of its subsequent co-formulation with ATV and DRV.

PARAMETER	RAL	EVG	DTG	BIC
Oral bioavailability %	ND	ND	ND	>70
Effect of food on AUC	⇧13–212%	⇧36-91 %	⇧33-36%	⇧24%
Plasma $t_{1/2}$, elim, h	9	9-14	14	18
Plasma protein binding, %	76–83	98–99	≥98.9%	99
Metabolism	UGT1A1	CYP3A > UGT1A1/A3	UGT1A1 >> CYP3A	CYP3A4 = UGT1A1
Renal excretion of parent drug, %	9	6.7	<1%	1
Cmax (ng/mL)	2170	1700	3670	9340
AUC (h*ng/mL)	6910	23000	53600	140 000
Cmin (ng/mL)	68.6	450	1110	3510
In vitro PA-$IC_{90/95}$ (ng/mL)	14.7 (IC_{95})	44.9 (IC_{95})	64 (IC_{90})	ND
In vivo EC_{90} (ng/mL)	ND	126	324	ND
IQ	8	10	17	16.2

Table 1.3: Pharmacokinetic properties of integrase inhibitors (reported mean values in adults with normal renal and hepatic function). Abbreviations: AUC: Area Under the Curve; $t_{1/2}$ elim: half-life of elimination; ND: not determined; UGT: UDP glucuronosyltransferase; IQ: inhibitory Quotient (C_{trough}/IC_{90} or IC_{95}), $EC_{50/90}$ concentration producing a 50/90% effect (reduction of HIV RNA) in vivo, $IC_{50/90/95}$ protein binding–adjusted concentration inhibiting viral replication by 50/90/95% in vitro.

1.2.3. Dolutegravir

1.2.3.1. DTG efficacy and PK

Approved individually in 2013 and in combination with two NRTIs as part of triple therapy in 2014, DTG was the first InSTI to be dosed once daily (OD) without boosting. It exhibits high efficacy, a predictable and favourable PK profile characterised by relatively low variability in licencing trials (C_{24h}, 25–26% CV) and excellent safety and tolerability profiles.[57-62] Overall, it stands relatively ahead of other ARVs and has now replaced EFV as first line agent in the World Health Organisation (WHO) guidelines, meaning it is likely to play a major role in the worldwide ARV scale up in coming years.[62-66] Additionally, a generic STR consisting of TDF/3TC/DTG is now available in some low to middle income countries (LMIC) at a median price of US$75 per person-year, making a DTG-containing regimen more

affordable than EFV-containing regimens and enabling progress towards universal access to HIV treatment.[66-68]

In the licencing phase, DTG was examined in five pivotal Phase III clinical trials, where it met criteria for superiority to EFV and to DRV/RTV in treatment naïve patients and to RAL in treatment experienced patients with at least two-class resistance but InSTI naïve. DTG also retained activity against some InSTI resistant viruses when dosed 50mg twice daily. **Table 1.4** summarises all five studies.[69-73] DTG dosing depends on patient-specific factors:[74]

- 50 mg once daily if patients are treatment naïve or treatment-experienced and InSTI-naïve
- 50 mg twice daily if patients also are taking a potent UGT1A/CYP3A inducer
- 50 mg twice daily if patients are InSTI experienced with associated InSTI resistance substitutions, or are suspected to be InSTI-resistant.

It is available alone as an individual tablet (Tiviquay®)[50] and co-formulated with abacavir/lamivudine (Triumeq®).[75] It is rapidly absorbed, achieving maximal blood concentration 2-4 hours after ingestion and has a terminal half-life of 12 hours, allowing OD administration without pharmacological enhancement.[59, 60, 76] There is minimal urinary excretion as it is metabolised predominantly through hepatic glucuronidation by UDP-glucuronosyltransferase 1A1 (UGT1A1) with a small contribution from cytochrome P450 3A4 (CYP3A4).[76, 77] DTG is detected in compartments such as CSF and cervicovaginal fluid at concentrations above that expected to confer continued antiviral efficacy and is therefore presumed to distribute widely.[78-80] Absorption of DTG is not pH dependent, food does increase the area-under-the-curve moderately (AUC ⇧33-66%) but it can be taken with or without.[81]

Trial name	Design	Duration	N	Regimen	Subject characteristics	Summary of results	Comments
SINGLE	Phase III, randomised 1:1, active control, double-blind, non-inferiority study	144 weeks	833	OD TDF/FTC/EFV *vs* OD DTG 50mg +ABC/3TC	ARV naïve Female: DTG 16%, EFV 11% VL >100,000 cpm: DTG 27%, EFV 24% CD4 <200 cells/mL: DTG 14%, EFV 15% EFV: n=412 (in ITT analysis) DTG: n=403 (in ITT analysis)	Efficacy (FDA snapshot): • Wk 48 HIV-1 RNA< 50 cpm: DTG 88% *vs* EFV 81% • Wk 96 HIV-1 RNA< 50 cpm: DTG 80% *vs* EFV 72% • Wk 144 HIV-1 RNA< 50 cpm: DTG 71% *vs* EFV 67% Criteria met for DTG superiority Superior efficacy primarily driven by fewer Discontinuations due to AE AEs: DTG 3%; EFV 11% CD4 increase at W144: DTG: 267, EFV 208 cells/ mm^3 Safety Insomnia only AE more reported DTG arm: 15 *vs* 10% GI symptoms and nasopharyngitis: 15-20% in both arms DTG: insomnia 10%, headache 6%, dizziness 7%	No treatment-emergent InSTI or NRTI resistance in DTG arm through to W144. In the EFV arm, 6 ppts developed NNRTI DRMs (K101E, K103K/N, and G190G/A) and 1 NRTI DRM (K65K/R) at time of failure
FLAMINGO	Phase III, randomized 1:1 open-label, active controlled non-inferiority study	96 Weeks	468	OD DRV/r 800/100mg *vs* OD DTG 50mg Each + TDF/FTC or ABC/3TC	ARV naïve Female: DRV 17%, DTG 13% VL >100,000 cpm: DRV 25%, DTG 25% CD4 <200 cells/mL: DRV 10%, DTG 10% DRV: n=242 (in mITT analysis) DTG: n=242 (in mITT analysis)	Efficacy (FDA snapshot): • Wk 48 HIV-1 RNA< 50 cpm: DTG 90% *vs* DRV 83% • Wk 96 HIV-1 RNA< 50 cpm: DTG 80% *vs* DRV 68% Criteria met for DTG superiority Virological non-response: DTG 5%, RAL10% Discontinuation due to AE: DTG 2%, DRV 4% CD4 increase at W48: DTG: 210, DRV 210 cells/ mm^3 Safety W96: The most common drug-related adverse events were diarrhoea DTG 10% *vs* DRV 24%, nausea DTG 13% *vs* DRV 14%, and DTG: insomnia 9%, headache DTG 7% *vs* DRV 15%, depression 5% dizziness 6%	Greatest difference in Wk96 viral suppression seen in pts with high viral load at baseline DTG 82% *vs* DRV 52%
SPRING 2	Phase III, randomised, 1:1 double-blind, active control, non-inferiority study	96 weeks	822	BID RAL 400mg *vs* OD DTG 50mg Each + TDF/FTC or ABC/3TC	ARV naïve Female: RAL 15%, DTG 14% VL >100,000 cpm: RAL 28%, DTG 28% CD4 <200 cells/mL: RAL 13%, DTG 12% RAL: n=411 (in ITT analysis) DTG: n=411 (in ITT analysis)	Efficacy (FDA snapshot): • Wk 48 HIV-1 RNA <50 cpm: DTG 88% *vs* RAL 85% • Wk 96 HIV-1 RNA <50 cpm: DTG 81% *vs* RAL 76% Criteria met for DTG non-inferiority Virological non-response: DTG 5%, RAL10% Discontinuation due to AE: 2% in each group CD4 increase at W96: DTG: 276, RAL 264 cells/ mm^3 Safety Similar AEs in both groups, (up to 13%) including nausea, headache, nasopharyngitis, and diarrhoea. DTG: headache 12%	Backbone similar in both arms: 60% TDF/FTC and 40% ABC/3TC. No DRM seen in the DTG arm In the RAL arm: InSTI: T97A, NRT:A62/K65R/K70/M184 M184I/A62V/M184V

SAILING	Phase II, Randomized 1:1double-blind, non-inferiority study	724 48 Weeks	BID RAL 400mg *vs* OD DTG 50mg Each + 2 additional ARVs with at least one fully active.	ARV experienced but InSTI naïve At least 2 class resistance Female: RAL 30%, DTG 34% VL >50,000 cpm: RAL 30%, DTG 30% PI/r in regimen: RAL 84%, DTG 85% RAL: n=361 (in ITT analysis) DTG: n=354 (in ITT analysis)	Efficacy (FDA snapshot): • Wk 48 HIV-1 RNA <50 cpm: DTG 71% *vs* RAL 64% Criteria met for DTG superiority Discontinuations due to AE: DTG 3%, RAL 4% CD4 increase at W96: DTG: 162, RAL 153 cells/ mm^3 Safety: Similar AE rates in both arms, (up to 9%); diarrhoea, URTI & headache commonest in both arms DTG: headache 9%	Fewer virological failure with treatment-emergent InSTI resistance in DTG arm (4 *vs* 17, p=0.003). DRMs in DTG arm FC <2 Those with DTG C_{trough} in the lowest quartile had the lowest virological response rates
VIKING 3	Phase III, single-arm open-label study	183 24 Weeks	BID DTG 50mg added to failing regimen Day 8, background regimen optimised with ≥1 fully active drug and DTG continued	ARV experienced with at least triple-class resistance (including INI resistance). VL > 500copies/mL Female: 33% VL >100,000 cpm: 22% Median VL: 4.38 (IQR), log10 cpm Median CD4 count: 140 cells/mL Median duration of prior ART: 14 years Median No of prior ARTs: 14	Efficacy (FDA snapshot): • Week 8: mean change in VL −1.43 log10 cpm • Wk 24 HIV-1 RNA <50 cpm: 69% Response was most reduced in subjects with Q148 + ≥2 resistance-associated mutation Discontinuations due to AE: 3% Safety: Drug related AEs rate: 15%, most common diarrhoea, nausea, and headache (similar to DTG 500mg OD)	Strong association between baseline DTG susceptibility and response n multivariate analyses Formed the basis of the FDA approval of DTG for INI-resistant patients, at BID 50mg

Table 1.4: Summary of the 5 pivotal licencing clinical trials for dolutegravir. Abbrieviations: DTG: Dolutegravir; TDF: Tenofovir Disoproxil Fumarate; FTC: Emtracitabine; EFV: Efavirenz; ABC: Abacavir; 3TC: Lamivudine; RAL: Rlategravir; DRV: Darunavir; OD: Once Daily; BID: Twice Daily; InSTI: Integrase Strand Transfer Inhibitor; PI/r: ritonavir boosted Protease Inhibitor; DRMs: Drug Resistance Mutations; ARV: Antiretrovirals; VL: Viral Load; cpm: copies per mL; AEs: Adverse Events; FDA: Food and Drug Association; FC: Fold Changes (in drug susceptibility); mITT: modified Intention To Treat analysis; GI symptoms: Gastrointestinal symptoms; IQR: Inter Quartile Range; Wk: Week; pts: patients; NRTI: Nucleoside Reverse Transcriptase Inhibitor

However, like other InSTIs, DTG is at risk of chelation and must not be ingested concomitantly with cations-containing agents. If co-prescription is inevitable, it should be administered 2 hours before or 6 hours after.[82] DTG holds a high genetic barrier.[76] It has an extended linker that allows its difluorophenyl group to enter further than other InSTIs into the pocket within the integrase active site, meaning it has a long dissociative half-life of 71h (*vs* 8.8 for RAL and 2.7 for EVG, $p < 0.0001$) and an off-rate 5 - 40 times slower than RAL and EVG.[83] Studies have also shown that it has the ability to adjust its structure and conformation in response to structural changes within the active sites of RAL- and EVG-resistant integrases, further raising its barrier to resistance.[84, 85] Finally, it has a high inhibitory quotient (IQ), determined by the remarkable distance of DTG minimum concentration within the dosing interval (C_{min}) from its half maximal inhibitory concentration (IC_{50}) at 50mg OD.[57] Importantly, no DTG resistance mutation (DRM) had been observed when the drug is used in first-line therapy, up until very recently.[86, 87] Major resistance pathways to InSTIs are shown in **table 1.5**. DTG distinguishes itself from both RAL and EVG by its very limited cross-resistance to these compounds.[88, 89] It is therefore a strong candidate for patients who struggle with adherence and **chapter 3** explores DTG PK forgiveness in order to inform physicians in prescribing it to poorly adherent patients. Three cases of first-line failure with mutations have now been reported worldwide.[90] Some potential resistance mutations (DRMs) to DTG have been identified at positions F121, S153, G118, E138, and R263 in vitro and in vivo.[91, 92] R263K was initially reported as the most common substitution in cell culture selections but it confers only moderate resistance to DTG (2.3-fold).[93] Overall, DTG associated DRMs decrease strand transfer activity and viral replication capacity, but these case reports highlight that this can be overcome and failing regimens do require a change of mode of action.[94-99]

Mutational pathways		Fold resistance		
		RAL	EVG	DTG
Y143 pathway	Y143C	<10	<2	<2
	Y143R	<50	<2	<2
	T97A, Y143C	>100	<2	<2
	T97A, Y143R	>100	<2	<2
	L74M, T97A, Y143G	<50	ND	<2
	L74M, T97A, E138A, Y143C	<20	ND	<2
N155 pathway	N155N	<50	<50	<2
	E92Q, N155H	<100	>100	<10
	L74M, N155H	<50	<50	<2
Q148 pathway	Q148H	<20	<10	<2
	Q148K	<100	<100	<2
	Q148R	<50	<100	<2
	E138K, Q148H	<10	<20	<2
	E138K, Q148K	>100	>100	<10
	E138K, Q148R	>100	>100	<10
	G140S, Q148H	>100	>100	<20
	G140S, Q148K	>10	<100	<2
	G140S, Q148R	>100	>100	<10
	E138A, G140S, Y143H, Q148H	>100	ND	<50
R263K pathway	R263K	<1	3	4
	R263K, H51Y	3-5	3	4-6
G118R pathway	G118R	10-17	>5	>8
	G118R, H51Y	ND	ND	ND
	G118R, E138K	4-20	4-5	8-13

Table 1.5: Major resistance pathways to raltegravir (RAL), elvitegravir (EVG), and dolutegravir (DTG). Abbreviations: ND: Not detetected. Adapted from Wainberg *et al.,* Can. J. Micr, 2017

Data post-marketing of DTG has focused on treatment simplification strategies, special populations infected with HIV, real life tolerability and DDIs, all of which are discussed below.

1.2.3.2. Treatment simplification

There has been interest, in recent years, in challenging the use of triple therapy, in order to lessen toxicity, cost and, potentially, drug interactions.[100-103] Well-designed, adequately powered long-term randomized controlled trials (RCTs) in diverse populations are needed to consider the widespread adoption of simplified regimen into clinical practice. At the time of writing, 12 initial studies investigating dual therapy

using DTG as the anchor drug had been published: 10 observational studies and 2 randomized trials.[104] Additionally, following supporting bioequivalence data, the first dual therapy STR, DTG/RPV (Juluca®) was approved by the FDA in 2018, for use in the US as maintenance therapy in selected patients.[54, 105]

1.2.3.2.1. Dual therapy

DTG + 3TC

ARV-naïve patients: In the single arm **PADDLE** study, 20 ARV-naïve patients initiating DTG/3TC OD achieved HIV-1 RNA <50 copies/mL at week 48 (bar 1 suicide, reported as unrelated to study drug).[106] This was followed up by another pilot study, the **ACTG5353**, which recruited 120 ARV naïve patients with HIV-1 RNA 1000-500,000 copies/mL and reported 90% achieving viral load (VL) <50 copies/mL at week 24 (FDA Snapshot). There were 3 protocol driven virological failures (VF) all of which had DTG levels reflective of suboptimal adherence.[107] Finally, a fully powered multicenter, parallel-group, double-blind, randomized phase III non-inferiority trial, **GEMINI 1&2**, compared DTG/3TC to triple therapy TDF/FTC/DTG in treatment naïve subjects with no major resistance associated mutations (n=1433). The FDA snapshot 48 weeks analysis reported non-inferiority of the dual therapy regimen (viral suppression 90% *versus* 93% in triple regimen), consistent across baseline HIV-1 VL and CD4 counts. There were no treatment-emergent InSTI or NRTI mutations observed in patients with confirmed VF.[108]

Pre-treated patients: The **ASPIRE** (randomized, phase III) and the **ANRS 167 LAMIDOL** (single-arm phase II) studies investigated safety and efficacy of DTG/3TC in pre-treated patients, reporting non-inferiority to triple therapy (91% *vs*

89%) in the **ASPIRE** study and 97% viral suppression in the **LAMIDOL** study, both at 48 weeks. There was no emergence of resistance in either study and no signal for lipid and renal toxicity.[109, 110] Since 3TC is already available in the generic form, this combination would be advantageous in LMIC, with caution applied to hepatitis B screening, as Hep B co-infection would require TDF, TAF or an alternative strategy.

DTG + RPV

RPV is the one NNRTI with no interaction with DTG.[111] This combination has mainly been studied as maintenance therapy, with the most compelling evidence coming from 2 large open-label randomized trials, **SWORD 1 and 2**, which are discussed in details in **chapter 4**. In summary, the pooled analysis showed non-inferiority to 3- or 4-drug regimen with improvements in bone, renal and lipid parameters, also confirmed in other studies.[112-116] There have also been similar findings in subjects with multiple previous treatment failures.[117]

DTG + DRV/RTV

Data for DTG/DRV/RTV dual therapy is discussed in detail in **chapter 4**. The **DUALIS** RCT demonstrated that OD DTG/DRV/RTV maintenance therapy, in suppressed patient, was non-inferior to continuing DRV-based triple therapy.[118] Additionally, cohort studies have investigated the use of DTG/DRV/RTV in multi-treatment experienced patients with resistance and all demonstrate viral suppression in >90% of subjects at 48 weeks and beyond, in this difficult to treat population.[119-121] Whilst an intensive 12-hour PK sub-study of the **DUALIS** trial was published, there is very limited PK data on DTG combined with co-formulated DRV/COBI and, to address this, **chapter 4** reports the intensive PK of DTG with and without DRV/COBI in healthy volunteers.

1.2.3.2.2. Monotherapy

Various small non-randomized studies have investigated the use of DTG monotherapy in selected ARV-naïve and experienced patients with conflicting results but with a positive response in some individuals.[101, 122, 123] This strategy is currently not recommended and requires further investigation before use.

1.2.3.3. Real life tolerability

DTG demonstrated excellent safety and tolerability in licensing trials.[61, 69-72, 124] However, real life data has emerged reporting higher than expected rates of adverse events (AE) and discontinuations secondary to AEs, particularly neuropsychiatric (NP) AEs and weight gain.[125-151] These data are discussed extensively in **chapters 1 and 6**. Consequently, there has been a call for DTG pharmacovigilance and for studies in diverse populations to further characterise those at risk of toxicity.[127] To this effect, the intensive pharmacokinetics of DTG in subjects over the age of 60 are described in **chapter 1** and DTG impact on sleep architecture and cognition is investigated in this population.

1.2.3.4. Special populations

Women

Women represent just over half of the worldwide HIV population and those of childbearing age represent a majority in certain LMICs; it is therefore important to characterise the use of newer ARVs in women.[1, 152] The phase IIIb randomised, open-label trial, **ARIA** showed superiority of the fixed drug combination (FDC) DTG/ABC/3TC over ATV/RTV plus TDF/FTC in 495 treatment-naïve adult women

over 48 weeks. Resistance mutations were low in those with VF and predictably, there was a higher rate of discontinuations in the ATV/RTV arm due to AEs (7% *vs* 4%).[153]

Pregnancy

The first PK evaluation of standard dose DTG in pregnant women, **IMPAACT P1026**, showed that the median DTG AUC_{0-24}, maximum concentration (C_{max}), and C_{24} were 25% to 51% lower in the second and third trimesters as compared with postpartum (n=29). These differences were deemed clinically non-significant since C_{24} remained above the DTG protein adjusted (PA)-EC_{90}, maternal viral loads remained undetectable, high placental transfer was demonstrated and all infants were HIV negative. For pregnant women initiating third trimester ART, the **DolPHIN-2** study confirmed that DTG-based therapy conferred quicker virologic response and increased likelihood of suppression at delivery than EFV-based therapy, with similar safety outcomes.[154] Of interest also, DTG transfers into breast milk, resulting in significant plasma concentrations in the infant.[155]

Safety data is available from over 10 observational studies and 1200 pregnancies including a number of monitoring databases, all of which suggest that there is no increased risk of preterm deliveries, small for gestational age or congenital anomalies in women started on DTG during pregnancy, relative to background risk.[156-160] But there is marked heterogeneity among the databases.[65, 161] And this year, some concerning results from an unscheduled analysis of the Botswana birth outcomes surveillance study revealed a signal for increased neural tube defects (NTD) in patients conceiving on DTG compared to other subgroups: 0.3% *vs* 0.12% in non-DTG ART at conception, 0.09% in HIV-negative women and 0% in DTG started after 1st trimester.[162, 163] As a result, warnings were issued from the WHO, FDA, European

Medicines Agency (EMA) and major associations and whilst the final data set from the Botswana study is awaited, most recommend avoiding DTG in women at risk of conception. [68, 164-167]

Renal Impairment

DTG is highly protein bound with low water solubility. There is no effect on exposure in mild to moderate renal impairment but unexpectedly lower DTG exposures were reported in HIV-seronegative subjects with severe renal impairment ($AUC_{0-\infty}$ 40% lower) and caution should be applied.[168] With regards to renal replacement therapy, Molto *et al.,* demonstrated minimal DTG removal by haemodialysis (extraction ratio 7%) suggesting no specific dosage adjustments required in this setting.[169-171] Notably also, DTG inhibits the renal organic cation transporters 2 (OCT2) on the basolateral membrane of renal cells, thereby causing a mild increase in serum creatinine in the first 6 weeks of treatment, this is not thought to be clinically significant.[57]

1.2.3.5. Drug interactions

In vitro, DTG was found to be a substrate for the efflux transporters P-glycoprotein (P-gp) and human breast cancer resistance protein (BCRP). It is metabolised by UGT1A1 and, to a lesser extent by CYP3A4 (10-15%), with minimal contribution from UGT1A3 and UGT1A9, without being an inducer or inhibitor of most of the usual metabolic systems, thereby limiting DDI perpetration.[57, 172] However, in a study using healthy volunteers, co-administration of DTG and metformin significantly increased metformin plasma exposure in a dose dependent manner; assumed to be OCT2 related. Dose adjustment and monitoring for lactic acidosis are therefore recommended in those at risk.[173] Studies investigating DTG interactions with the anti-mycobacterial agents initially showed that co-administration with the potent inducer

rifampicin, required twice daily dosing of DTG 50 mg.[174] More recent intensive co-administration PK data showed that DTG dosing could be simplified to 100mg OD.[175] A dose adjustment is not necessary with rifabutin 300 mg.[176] There does not appear to be a significant interaction between DTG and oral contraceptive pills or proton pump inhibitors.[50] However, antacids and supplements containing divalent cations, can attenuate DTG absorption through chelation and DTG should be taken 2 hours prior to or 6 hours after.[82]

Amongst the ARVs, both EFV and ETV significantly lower DTG levels and should be avoided unless ETV is administered with RTV.[177, 178] Finally, the PIs currently used can be safely administered with DTG, when boosted with RTV. However, interactions between DTG and COBI are unclear and require further investigation.[179] To address this gap in knowledge and as previously mentioned, **chapter 4** investigates the pharmacokinetics of DTG with and without co-administration of DRV boosted with COBI.

1.2.4. Bictegravir

Bictegravir, the fourth InSTI was recently licenced, co-formulated with FTC and TAF (BIC/F/TAF, Biktarvy®). It was approved in Europe in June 2018, for adults infected with HIV-1, without evidence of viral resistance to InSTIs, FTC or TDF.[180] Like DTG, it has high genetic barrier to HIV-1 resistance.[181] In phase 3 trials, it was non-inferior to DTG-based therapy in treatment-naïve adults through to 96 weeks and, similarly, was noninferior to ongoing DTG/3TC/ABC or boosted EVG- or PI-based therapy in preventing virological rebound over 48 weeks in treatment-experienced patients. No resistance emerged to any of the antiretrovirals in the STR.[182-187] It is generally well

tolerated, requires no prior *HLA-B*5701* testing (unlike Triumeq®), fulfils the ARV regimen requirement for patients with HBV co-infection (TAF) and can be used in renally impaired patients with creatinine clearance ≥ 30 mL/min.[181, 188]

1.3. COBICISTAT

For over a decade, low dose RTV has been used as a pharmacological booster for most PIs, in order to increase the half-life ($t_{1/2}$), the time to reach C_{max} (t_{max}), C_{max}, and AUC through potent CYP3A4 and P-gp (intestinal and hepatic) inhibition. Cobicistat (COBI) was released in 2014 as an alternative to RTV, available either as a single agent (Tybost®) or co-formulated with the new integrase inhibitor at the time, Elvitegravir (EVG/COBI/FTC/TDF, Stribild®).[189, 190] It's approval followed phase III studies demonstrating non-inferiority of EVG/COBI/FTC/TDF to EFV, ATV/RTV and RAL-based regimen in treatment naïve and virologically suppressed ARV-experienced participants (**GS-102, 103, 145, STRATEGY-NNRTI and STRATEGY-PI).**[191-195] COBI is a structural analogue of RTV. It inhibits CYP3A4 with a similar potency and, to a lesser degree, P-gp.[196-201] Its tolerability profile overall is similar to that of RTV.[199] At a dosage of 150 mg OD, it provides bioequivalent exposures of atazanavir (300 mg OD) and darunavir (800 mg OD) compared with those observed with 100 mg of RTV OD and similar virological suppression when compared to ATV/RTV.[201-211] COBI does not have antiviral activity and good solubility lends it to co-formulation. It was approved co-formulated with ATV (Evotaz®) and DRV (Rezolsta®) in 2015, potentially reducing pill burden in patients requiring a boosted PI. The PK properties of COBI are summarised in table **1.6**; there are key pharmacological differences between RTV and COBI. Unlike its counterpart, COBI does not inhibit CYP1A2, CYP2C8, CYP2C9 or CYP2C19 and is a weaker

inhibitor of CYP2D6 and CYP2B6.[197-199] The greatest discrepancy between the two, however, lies in the fact that COBI does not induce the activity of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or UGT1A1 and is not expected to induce that of CYP3A4 or P-gp.[198-201] Additionally, compared to RTV, COBI has a limited effect on the pregnane X receptor (PXR), a nuclear receptor activated by xenobiotics that subsequently induces and regulates the expression of various drug-metabolising enzymes, including CYP 450 and UGT.[197, 198, 212] This is particularly salient to patients switching from an RTV-boosted to a COBI-boosted regimen, when the loss of CYP/glucuronidation induction may potentially require the dose adjustment of co-prescribed drugs.[198]

PARAMETER	COBICISTAT
Oral bioavailability %	ND
Effect of food on AUC	⇧*
Plasma $t_{1/2,}$ elim, h	3-4
Plasma protein binding, %	97-98
Metabolism	CYP3A4 >> CYP2D6
Renal excretion of parent drug, %	8.2
Cmax (ng/mL)	1200
AUC (h*ng/mL)	10 900
Cmin (ng/mL)	70

Table 1.6: PK properties of cobicistat (reported mean values in adults with normal renal and hepatic function). Abbreviations: AUC: Area Under the Curve; $t_{1/2}$ elim: half-life of elimination; ND: not determined. * no formal study conducted

No COBI dose adjustment is required in patients with renal impairment as data shows its PK are not significantly changed following administration of standard daily doses in subjects with severe renal impairment (eGFR <30 mL/ min).[213] COBI, however, like RTV, does inhibit tubular secretion of creatinine leading to an apparent reduction in estimated creatinine clearance; yet actual glomerular filtration rate, as measured by the clearance of iohexol (a probe excreted solely by glomerular filtration) is not altered.[214] The effect is reversible and mainly reflects the inhibition of creatinine

secretion by the multi-antimicrobial extrusion protein 1 (MATE1) transporter, on apical proximal tubular cells.[214] Interestingly, COBI leads to greater creatinine changes than RTV despite similar IC_{50} values for MATE1, which may relate to active OCT2-mediated COBI transport into tubular cells, increasing its availability to MATE1.[198, 215] Caution, however, remains when COBI is co-administered with and boosts potentially nephrotoxic drugs such ATV and TDF. COBI dosage adjustment is not required in patients with mild to moderate hepatic impairment, however is not recommended in patients with severe hepatic impairment (lack of data).[199] To address some gaps in the literature and inform clinicians considering the use of COBI in commonly encountered clinical situations, **chapter 3** establishes the PK forgiveness of Evotaz® and Rezolsta® following cessation of drug intake and compares it to that of RTV-boosted ATV and DRV. In addition**, chapter 5**, characterises the PK of one of the commonest contraceptive pill, ethinylestradiol/levonorgestrel, when it is co-administered with ATV/COBI.

1.4. GENETIC VARIATION AND ARV PK/PD

The penultimate chapter of this thesis, **chapter 6**, focuses on the pharmacogenetics of dolutegravir PK. Background on pharmacogenetics is provided in the section below.

The inter- and intra-individual variability observed in both therapeutic and toxic effects of a drug is governed by demographic, physiological and genetic factors. Pharmacogenomics is the study of the contribution of naturally occurring genetic variants to this variability whilst pharmacogenetics refers to single drug–gene interactions.[216] Precision medicine, then aims to use pharmacogenomic/enetic data, amongst other parameters, to predict a patient's clinical outcome and to tailor therapy to genetically-defined sub-populations.[217] This is particularly important in areas of

medicine, where complex and potentially toxic therapies are prescribed in multiples and over prolonged periods of time such as in HIV.[216] Genetic sequence variations between individuals include single-nucleotide polymorphisms (SNPs), insertions and deletions (indels) and short tandem repeats (STRs) amongst others and are described as common if they exist in individual genomes at frequencies > 5%. SNPs are the most frequent (>90% of all sequence variations).[218] They can result from either the deletion, insertion or substitution of a single nucleotide in a sequence and occur either within the protein coding (exons) or non-coding (regulatory or intron) regions of genes or within intergenic regions. SNPs within coding region that result in the same amino acid sequence being translated are called synonymous whilst SNPs that result in a different amino acid or a premature stop codon are called nonsynonymous. Nonsynonymous SNPs can either be classified as missense (leading to change in one amino acid in a protein) or as nonsense (leading to a premature stop codon and protein truncation), both potentially altering the level of expression and/or intrinsic activity of metabolically active proteins.[216, 218] Importantly, SNPs in non-protein-coding regions may still have a pharmacological effect if they disrupt the regulatory functions of these regions, such as transcription regulation.[219, 220] SNPs are usually associated via haplotype groups, which occur because multiple SNPs are inherited together. This is referred to as linkage disequilibrium, which is the non-random association of alleles on the same chromosome within a particular population.[217, 218, 221]

As PK and biodistribution are inherent to both drug safety and efficacy, the most commonly studied variants in HIV are SNPs in genes implicated in drug absorption, distribution, metabolism and excretion pathways (ADME, **figure 1.8**), and include SNPs in genes that are involved in the production of transporters and enzymes.[217] In

addition, there is also increasing interest in nuclear receptors that regulate the expression of ADME genes (such as PXR), in human leukocyte antigen (HLA) subtypes involved in hypersensitivity reactions (HSR) and in genes implicated in the development of metabolic toxicity.[222]

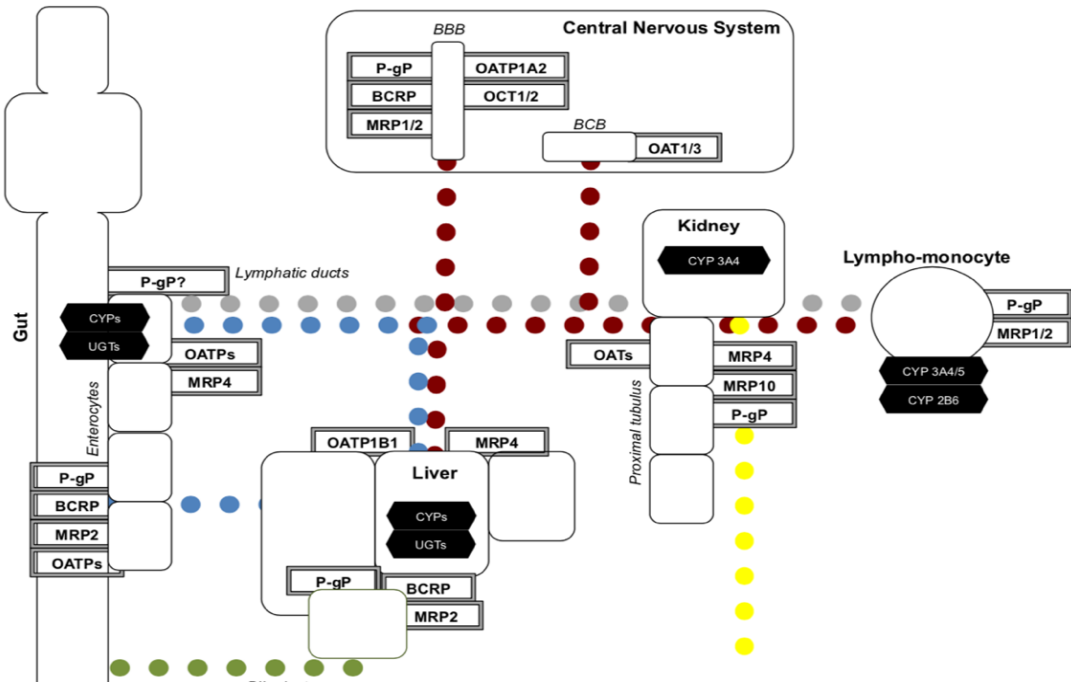


Figure 1.8: Enzymes and transporters involved in drug disposition. Blue & Red dots: venous & arterial circulation. *BBB*: Blood Brain Barrier, *BCB*: Blood Cerebrospinal fluid Barrier. From Calcagno *et al.* 2017

The completion of the Human Genome Project in 2001, heralded the beginning of great technological advancements in the era of pharmacogenetics and precision medicine. Two main approaches have been used to study pharmacogenetics: 1) the analysis of single gene variant's effect after mechanistic observations and 2) genome-wide association studies (GWAS). The first relies on preceding observations and understanding of drug metabolisms and transports and the latter tests the effect of a large number of genes on a selected target, then potentially requires post-hoc confirmatory in vitro studies.[222] The study described in **chapter 6** uses a single gene

variant approach following careful selection of common SNPs related to enzymes and transporters that are known to be involved in the disposition of DTG.

It is important to note that the effect of genetic variants on drug disposition might vary according to the molecule characteristics and metabolism. An accurate selection of the compound to be studied might be cost effective. For instance, a practical and relatively straightforward method called the relative genetic contribution (rGC) has been proposed and recently applied to ARVs. It is quantified comparing the intra- and interpatient variation in PK parameters following the repeated administration of a drug; compounds with higher rGC have a higher genetic contribution to interpatient variability, warranting PK/PG analysis. ARV class-specific differences in rGC may exist with NNRTIs seemingly ranking the highest and RAL the lowest.[223]

The last decade has seen a surge of data in HIV pharmacogenomics, suggesting that the choice and dose selection of ARVs might be improved upon knowledge of a patients' pharmacogenetic background;[216, 217, 222] however, findings have frequently been conflicting and very few confirmatory clinical studies have been published. Only three pharmacogenetic markers have been widely replicated and have attained the point of potential practical utilisation in the clinical setting: HLA-B*5701 (ABC HSR), *CYP2B6* 516G>T (EFV and NVP PK and toxicity) and *UGT1A1**28 (ATV hyperbilirubinaemia). In practice, only data on HLA B*5701 genotyping in abacavir candidates is clinically decisive and routinely used.[224, 225] Limits to the detection and strength of any genotype-phenotype relationship include the fact that several enzymes and transporters are involved in the ADME of any one drug, often with complex

interactions between them. Additionally, huge variability in genetic variants exists among different ethnic groups meaning ethnic stratification of data is also key.[217, 222]

DTG and COBI (in its role as a pharmacological booster for EVG, ATV and DRV) are the focus of this thesis; whilst there are pharmacogenetic data for the former, no study has been published on the latter. DTG pharmacogenetic studies are discussed in details in **chapter 6**. In summary, studies have primarily investigated the association between DTG PK and *UGT1A1* and *ABCG2* variants. Approximately 40-70% of all clinical drugs are metabolised by UGTs within humans and there are three main subfamilies (UGT1A, UGT2A and UGT2B).[222, 226] UGT1A1-mediated glucoronidation is the main metabolic pathway for DTG.[57] Several genetic variants in the UGTA1 gene have been identified, the most common is a thymidine and adenine (TA) repeat polymorphism in the promoter region. Increasing number of TA repeats is inversely associated with UGT1A1 transcription, with five and six repeats (alleles *36 and *1, respectively) being associated with increased and normal UGT1A1 activity, and seven and eight repeats (*28 and *37, respectively) with low UGT1A1 activity.[227-229] Individuals are categorised as extensive, intermediate or poor metabolisers according to their allele combination.[222, 229] *UGT1A1**6 211G>A is also a common variant associated with reduced enzyme function, it is most prevalent in individuals of Asian descent.[229] *Chen et al.* were the first to suggest a moderate relationship between *UGT1A1**28 and *6 and DTG PK, publishing at the time of DTG FDA approval. This was later supported by data from Yagura *et al*.[230,148] As previously mentioned, DTG is a known BCRP substrate and the homozygous *ABCG2 c.*421C>A (rs2231142) allele, coding for BCRP, has been associated with a 50% higher DTG C_{max}.[231]

Since there have been concerns around high rates of insomnia and NP-AEs with DTG use, Yagura *et al.* suggested an association between the *UGT1A1* poor metabolising status and C_{min}-mediated NPAEs as well as all AE-related DTG cessations in a Japanese cohort.[147, 148] Finally, Borghetti *et al.* recently reported an association between the *SLC22A2* 808C>A (rs316019) variant, coding for OCT2, and a set of sub-clinical neuropsychiatric measurements in a European cohort.[232] However, findings from both groups have yet to be reproduced and collinearity between the respective genetic variants and high DTG concentrations needs to be clearly distinguished.

Chapter 6 therefore aims to address some existing gaps in currently published data and confirm some already published findings, through exploring the role of *UGT1A1*, *ABCG2*, *CYP3A* and *NR1I2* SNPs on plasma DTG concentrations, in isolation or in combination, in pooled subject data from four clinical trials investigating the PK of 50mg DTG taken OD.

1.5. THESIS OBJECTIVES

As discussed above, 2013-14 saw the approval of two significant new agents for the treatment of HIV, DTG and COBI. Both addressed unmet clinical needs at the time and are now widely used. DTG's properties have secured its place as a preferred agent in major guidelines, including the WHO Guidelines, meaning that by 2025, an estimated 15 million PLWH could be taking it.[7, 63, 65] This raises a number of clinical questions about the pharmacological behaviour of newly approved drugs in real-world settings. Often, these questions are not addressed in licencing trials, because they are not FDA and EMA requirements for approval and RCT participants often differ substantially from broader patient populations.[127] Therefore, in this thesis, we aimed

to investigate common and important clinical pharmacological scenarios reflective of HIV treatment in real life, focusing particularly on PK in older populations, PK forgiveness for the management of late and missed doses, co-administration with common drugs and genetic determinants of PK.

More specifically, **Chapter 2** characterises the intensive PK of DTG in HIV infected participants over the age of 60 and its pharmacodynamic effect on sleep over 6 months. The PK tails of DTG and COBI-boosted EVG, ATV and DRV are then reported in **chapter 3**. **Chapter 4 and 5** investigate the effect of co-administration of common drugs, with DTG and COBI-boosted DRV in **chapter 4** and the oral contraceptive pill (OCP) and COBI-boosted ATV in **chapter 5.** Finally, **chapter 6** explores the impact of genetic variability in drug disposition genes on the PK of DTG.

CHAPTER 2

Increased Dolutegravir Peak Concentrations in People Living With HIV Aged 60 And Over And Analysis of Sleep Quality And Cognition

CITATION

Elliot ER, Wang X, Singh S, Simmons B, Vera JH, Miller RF, Fitzpatrick C, Moyle G, McClure M, Boffito M. Increased dolutegravir peak concentrations in people living with HIV aged 60 and over and analysis of sleep quality and cognition. *Clinical Infectious Diseases*. 2018 May 16. doi: 10.1093/cid/ciy426.

CONTENT

2.1. INTRODUCTION

By 2015, one in three people accessing HIV care in the UK[233] and almost half in the US were aged 50 and over.[234-238] With advancing age, several physiological changes are known to occur and affect drug pharmacokinetics and pharmacodynamics.[239]

DTG is now the drug of choice for many HIV providers, thanks to high efficacy firmly demonstrated in trials, retained activity against some InSTI-resistant HIV-1 phenotypes and low potential for drug-interactions.[72] Overall, these properties make it a strong candidate for therapy in older PLWH.[236] As discussed in **chapter 1**, DTG demonstrated favorable safety and tolerability profiles in pre-marketing trials with a <2% discontinuation rate secondary to any adverse events (AEs), which was comparable to RAL and superior to EFV.[69, 240] However, contrasting real-life data from cohort studies, involving >6400 patients overall, have revealed unexpectedly higher discontinuation rates secondary to any AEs (2.3-13.7%, median time 72 days), most commonly due to gastrointestinal AEs and insomnia/sleep disturbances and other NP-AEs (mean incidence 3.5%, range, 1.4-7.2%), regardless of prior neuropsychiatric history, thereby implicating a potentially neurotoxic effect of DTG. Some attempts at defining risk factors for NP-AEs, including PK and pharmacogenetics have been made but data remain conflicting.[127-139, 146-149] Comparison studies suggest that NP-AEs are more common with DTG than other InSTIs.[135-138] Interestingly, in several reports, NP-AEs and DTG discontinuation were significantly higher in women and in PLWH >60 years old, two groups which are often under-represented in licensing trials due to highly selective inclusion criteria.[133-135] This has prompted a call in the literature for prospective studies to evaluate DTG-associated NP-AEs, using detailed, longitudinal,

validated sleep architecture and neuropsychological analyses, in conjunction with PK determinations, particularly in special populations.[241]

Importantly, also, a high prevalence of sleep disturbances is already described in the HIV population, even in the cART era, (30-73% *vs* 10-20% in the general population)[242, 243] and it is strongly associated with poorer disease outcomes, cognitive impairment and HIV-associated dementia.[242, 244] It is, therefore, important to characterise the role of aging on DTG PK/PD, especially with regards to central nervous system (CNS) toxicity and sleep disturbances. The primary objectives of this study were to describe the steady state PK of DTG 50 mg OD in PLWH ≥60 years and compare them to a published younger population (from the SINGLE trial).[69] The secondary objectives were to evaluate, in detail, changes in sleep and cognition over six months following a switch from non-DTG-based ARV regimen to ABC, 3TC and DTG, as a fixed dose combination tablet.

We hypothesised that age-related changes in drug PK might impact DTG, except its metabolism since it is mainly by UGT1A1 and no evidence supports age-related glucuronidation changes.[245] We also expected a reverse-association between sleep/cognition changes and PK parameters, particularly at the high end of the therapeutic range (or higher).

2.2. METHODS

2.2.1. Participants

Written informed consent was obtained from male and female PLWH, stable on cART, aged ≥60 years with a body mass index (BMI) 18-35 kg/m^2. The protocol required that

approximately 70% of subjects be ≥65 years (to ensure a variable age range). Eligibility criteria included plasma HIV-RNA <50 copies/mL at screening and no history of treatment failure or documented significant drug resistance on viral genotyping. With ABC use, a negative HLA-B*5701 allele result was required and participants were screened for cardiovascular (CV) risk using the QRISK2 calculator[56, 246] (eligible if 10-year risk of CV event was <20% or if risk factors were well controlled with medication/lifestyle measures). Participants were excluded if they had: significant acute/chronic illnesses; abnormal physical examination, ECG or laboratory determinations or use of known interacting drugs/remedies. No patients had preceding Primary Sleep Disorder diagnoses. The study was approved by the London Central Research Ethics Committee and the Medicines and Healthcare products Regulatory Agency (MHRA) and ran in accordance with Good Clinical Practice and the Declaration of Helsinki (NCT02509195).

2.2.2. Study design

This was a four-centre, 180-day (excluding screening and follow-up), open-label, prospective PK/PD study. After screening, eligible subjects were switched to ABC/3TC/DTG 600/300/50 mg FDC (Triumeq®)[75] on day 1, one pill OD, orally, in the morning with or without breakfast for the study period, except on day 28 (D28). On D28, subjects underwent intensive DTG PK determinations, having fasted for six hours pre-dose and four hours post-dose to match the SINGLE PK sub-study circumstances.[69] Blood samples were collected pre-dose, 1, 2, 3, 4, 8, 12- and 24-hours post-dose. Study medications safety was evaluated using the National Institute of Allergy and Infectious Diseases (NIAID) Division of AIDS table for grading the severity of adult and paediatric adverse events (2004), that characterises abnormal

findings, vital signs, physical examinations and clinical laboratory investigations. HIV viral load was checked on all safety and PK visits. Medication compliance was assessed through direct questioning and pill count.

2.2.3. Collection and quantification of plasma dolutegravir

Whole blood samples were collected at each time-point on D28, from an indwelling venous catheter, into 6 mL spray-coated EDTA tubes. Following centrifugation, plasma was aliquoted equally into three 2.0mL tubes (Sarstedt, Germany) and stored at -80^{o}C. Samples were then shipped on dry ice to the Jefferiss Trust Laboratory (Imperial College London). DTG plasma concentrations were determined using ultra-performance liquid chromatography (UPLC) coupled with UV detection.[247] The assay calibration range was 0.25-10 mcg/ml, intra-assay variability 3.3%-6.1% and inter-assay variability 4.5%-5.7%. Overall accuracy was between 90.7% and 97.7% for three different quality control sample concentrations. The laboratory adheres to the ARV International Inter-laboratory Quality Control Program.[248]

2.2.4. Pharmacokinetic and statistical analysis

A sample size of 40 subjects was calculated to provide at least 80% power to detect DTG PK parameter changes in older people against 16 controls. The calculated parameters were plasma concentration measured 24 hours after the observed dose (C_{24}), maximum observed plasma concentration (C_{max}), area-under-the-plasma-concentration-curve from 0 to 24 hours ($AUC_{0–24}$) and half-life ($t_{1/2}$). All PK parameters were calculated using actual blood sampling time and non-compartmental modelling techniques (WinNonlin-Phoenix, version 7.0). Descriptive statistics, including geometric mean (GM), 95% confidence interval (CI) and percentage coefficient of variation (CV% = 100*standard deviation/mean) were calculated for

DTG PK parameters at all time-points on D28, and compared to those obtained from the SINGLE PK sub-study control HIV population (≤50 years, n =16)[69] using the non-parametric Mann-Whitney U test.

2.2.5. Sleep and cognitive data collection

Six published and validated self-reported paper questionnaires (**table 2.1**),[249-254] recording different aspects of sleep, were administered to participants at baseline and on days 28, 90 and 180 in order to provide a comprehensive description of sleep quantity, quality, impact on daytime function, wakefulness, mental status and general wellbeing before and after the medication switch. Answers to each question were coded as per questionnaire protocols (appendix 2) and entered into Excel for scoring. Neurocognitive testing was carried out on D1 and D180 using the validated, widely used Cogstate® computerised assessment software,[255] which evaluates a range of cognitive functions through eight domains: detection (DET)/identification (IDN) (speed of performance); one card learning (OCL), one back memory (OBM)/two back memory (TWOB) (accuracy of performance); Groton Maze learning (GML), Groton Maze recall (GMR), and set-shifting (SETS) (number of errors made on testing). Participants completed a mock practice at screening to minimise learning effect.

2.2.6. Sleep and cognitive data analysis

Sleep baseline characteristics and outcome measures at each time-point were descriptively summarised using medians, interquartile ranges (IQR), and proportions. Composite scores for sleep questionnaires were calculated and interpreted as per questionnaire protocols and cut-offs (**table 2.1**). Neurocognitive scores were analysed using Cogstate® recommendations.[256] Changes in cognitive scores were calculated for each subject for each domain (baseline-D180), and were standardised according to the

within-subject standard deviation (WSD). The score sign was reversed where appropriate so positive values represent improvement for all domains. A composite score for the change from baseline was calculated by averaging the standardised change scores across all Cogstate® tasks for each individual. As data was not normally distributed, non-parametric tests were used for analysis. Changes in sleep and cognitive scores from baseline to each time-point were tested for significance using the Wilcoxon sign-rank test. Spearman's correlation examined correlations between outcomes and DTG PK parameters.

As EFV use is associated with NP-AEs, especially sleep disturbances, a sub-analysis was conducted using the Mann-Whitney test to compare individuals who switched from an EFV-based regimen to those who didn't, thereby preventing EFV removal from potentially masking DTG effects.[56]

Internal consistency was evaluated for outcomes with multiple domains using Cronbach's α and corrected component-total Spearman's rho (r_s) correlations ($\alpha \geq .70$ and $r_s \geq .30$ indicated adequate internal consistency). Correlation between different sleep questionnaires was evaluated at baseline to determine the level of agreement.
Statistical analyses were performed using Stata (version 14.1) and GraphPad Prism (version 7.03). In the analyses, p-values, uncorrected and corrected for multiple comparisons, were calculated; $p < 0.05$ was deemed significant.

The Bonferroni correction was used to account for multiple comparisons.

Questionnaire	Process	Main Domains	Recall period	No of items	Time to fill (mins)	Scores
PSQI	Self-Reported 0-3 Likert scale	Sleep Quality, sleep Disturbance and sleep habits	1 mth	19	5-10	Score of 5 or more indicates poor sleep quality Global score calculated by summing subscale scores (not calculated for individuals with missing results)
ESS	Self-Reported 0-3 Likert scale	Level of sleepiness/ propensity of falling asleep	N/A	8	< 5	≥11 indicates excessive daytime sleepiness
FOSQ	Self-Reported 0-4 Likert scale	Functional impairment in activities of daily living resulting from sleepiness	N/A	30	15	5 domains: for each domain, lower scores indicate more acute issues. Each domain score calculated by averaging answered domain questions. Global score calculated by averaging the subscale scores & multiplying by 5 (allows for missing subscale scores)
ISS	Self-Reported 0-4 Likert scale	Nature, severity and impact of insomnia	2 wks	7	<5	0-7 no insomnia 8- 14 subthreshold insomnia 15-21 moderate insomnia 22-28 severe insomnia
FSS	Self-Reported 1-7 Likert scale	Effect of fatigue on motivation, exercise, physical, social and family functioning	1 wk	9	<5	>5 indicates abnormal fatigue
SDQ	Self-Reported 1-5 Likert scale	Sleep quality Sleep disturbance Daytime function Medication Medical family history	6 mths	175	30	4 sleep disorders categories: Sleep Apnoea Syndrome, Narcolepsy, Periodic Limb Movements Disorders and Psychiatric sleep disorders.
Cogstate neuro-cognitive test	Computerised battery	Detection Identification Set Shifting Groton Maze Learning Groton Maze Recall One Card Learning One Back Memory Two Back Memory	N/A	8 tasks		Score provided for each of 8 domains using optimal outcome measure (as defined by Cogstate guidelines). Composite score for change from baseline calculated by averaging standardised change scores

Table 2.1: Summary of content, process and scoring of sleep questionnaires and cognitive testing. Questionnaire acronym definitions available listed in text

2.3. RESULTS

2.3.1. Study population

Fifty-three subjects were screened; 43 enrolled and received at least one study drug dose. Three/43 participants withdrew before D28 and could not be included in the PK analysis (two moved abroad and one experienced fatigue and photosensitivity attributed to the study drugs). Forty participants completed the PK phase and 38 attended the final study visit (D180). One participant withdrew secondary to insomnia/vivid dreams (resolved by switching to TDF/FTC/RAL) and the other withdrew for job relocation; both were included in D28 PK and PD analyses. Subject and control characteristics are summarised in **table 2.2**.

2.3.2. Dolutegravir plasma pharmacokinetics

Steady-state PK parameters are summarised in **table 2.3**; **figure 2.1** demonstrates GM DTG concentration *vs* time curves for the observed and control populations. There were no differences in DTG $AUC_{0\text{-}24}$, C_{24} or $t_{1/2}$ between the two populations. However, C_{max} (approximately two hours post-dose in both groups) was significantly higher in subjects ≥60 years old (GM 4246 *vs* 3402 ng/mL, p=0.005). The PK parameters for the participant who withdrew secondary to NP-AEs after day 28 were: C_{max} 5300 ng/mL, C_{24} 2013 ng/mL, $AUC_{0\text{-}24}$ 77942 hr*ng/mL and $t_{1/2}$ 19.8 hrs; all were above the 95th percentile for the study group.

	Variable	Study subject in PK analysis (n=40)	Controls (n=15)
Age	Median (range) in years	66 (60-79)	36 (22-50)
Ethnicity (n)	White British/Irish/Other	33	11
	African Heritage	3	3
	Hispanic	2	0
	Asiatic	2	0
	American Indian/Alaskan Native	0	1
Gender (n)	Male	39	15
	Female	1	1
Pre-switch regimen			
Backbone (n)	ABC/3TC	16	N/A
	TDF/FTC	20	N/A
3rd Agent (n)	Boosted Protease PI (of which mono & dual therapy with RAL)	9 (2 & 1)	N/A
	NNRTI (of which EFV)	24 (17)	N/A
	RAL (of which dual tx with PI/b)	6 (1)	N/A
	AZT	1	N/A
Salvage tx (n)	FTC, MVC, DRV, RTV	1	N/A

Table 2.2: Demographic and clinical characteristics of study participants and controls. Tx: therapy

	Observed group (n=40)				Control group (n=15)				P value (Mann-Whitney U)			
	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-24} (ng.h/ml)	$t_{1/2}$ (hrs)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-24} (ng.h/ml)	$t_{1/2}$ (hrs)	C_{max} (ng/ml)	C_{min} (ng/ml)	AUC_{0-24} (ng.h/ml)	$t_{1/2}$ (hrs)
GM	**4246**	1052	51799	12.84	**3402**	942	48068	14.35	**0.005**	0.772	0.56	.706
Low 95%	4018	999	49405	12.05	3008	799	42350	11.16	-	-	-	-
Up 95%	4767	1351	59020	14.93	4030	1461	59898	21.44	-	-	-	-
CV %	27	48	29	34	29	58	34	62	-	-	-	-

Table 2.3: DTG steady-state PK parameters for the observed and control groups over 24 hours. In bold: significant difference in C_{max}

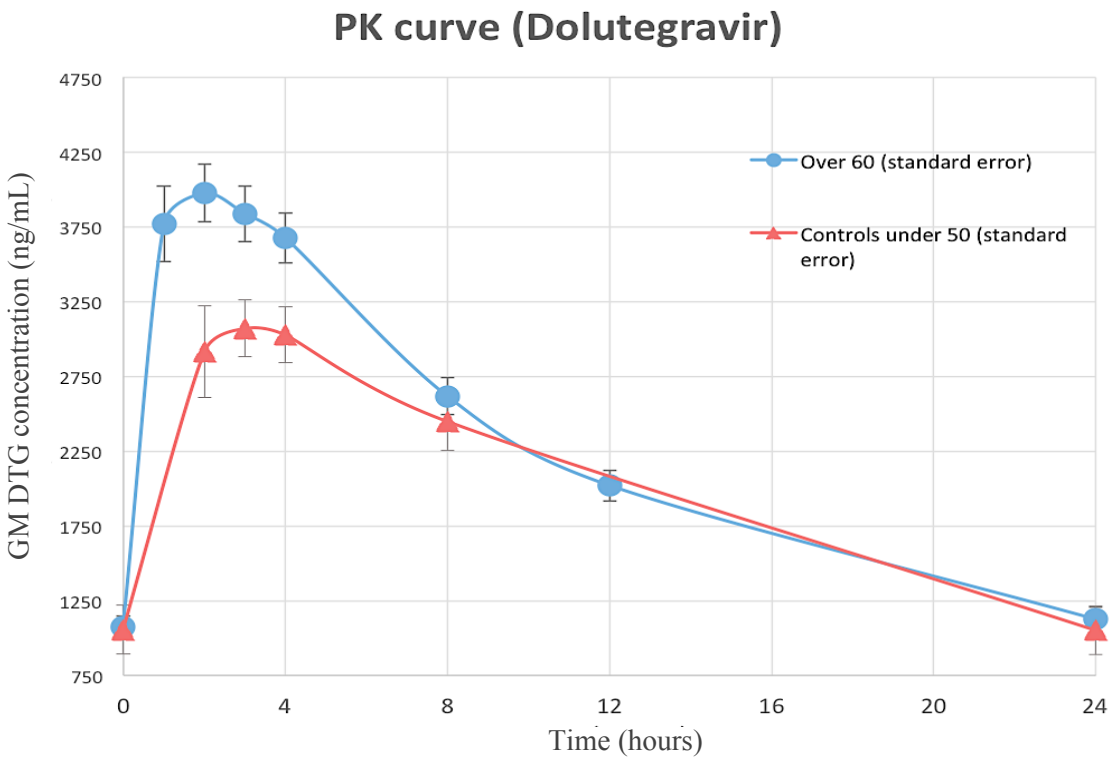


Figure 2.1: GM DTG concentration *vs* time curves for the observed and control populations

2.3.3. Sleep questionnaire results at baseline and follow up

Detailed response rates and median (IQR) scores per questionnaire, domain and time-point are in **appendix 2: table A and figure B.**

2.3.3.1. Overall sleep impairment: *Pittsburgh Sleep Quality Index (PSQI)*[249]

Median global PSQI score was higher at D28 *vs* baseline (5.0 *vs* 6.0, p=0.02 adjusted for multiple testing) but at no other time-points. No domain achieved statistical significance individually. Internal consistency was acceptable for the global score (α=0.72). Corrected component-total correlations ranged from 0.19 (daytime dysfunction) to 0.66 (quality).

2.3.3.2. Insomnia: *Insomnia Severity Index (ISI)*[250]

Median (IQR) global ISI scores remained stable (range 5-6.5); four individuals developed moderate insomnia over time (ISI 14-21; not significant) and one subject's severe insomnia (ISI >21) improved whilst another's developed by D28 leading to discontinuation (participant described above).

2.3.3.3. Daytime sleepiness: *Epworth Sleepiness Scale (ESS)*[251]

At baseline, 29% individuals were considered 'sleepy' (ESS >10) compared with 24% at D180 (not significant).

2.3.3.4. Daytime function: *Functional Outcomes of Sleep Questionnaire (FOSQ)*[252]

Median (IQR) global FOSQ remained stable from baseline to day D180 (range 18.01-18.81/20) with a generally good level of daytime function across the cohort.

2.3.3.5. Fatigue severity: *Fatigue Severity Scale (FSS)*[253]

At baseline, 4/39 (10%) individuals reported having fatigue; this was 20% on D180 (not significant).

2.3.3.6. Risks of possessing sleep disorder: *Sleep Disorder Questionnaire (SDQ)*[254]

No participants met the diagnostic criteria at baseline for any of the four sleep disorders tested and no significant change in scores was observed over time.

2.3.3.7. Correlation between sleep measures

There was a significant correlation between all sleep measures evaluated by more than one questionnaire across all scores at baseline ($0.37<r<0.83$; $p<0.05$).

2.3.3.8. Sleep scores by EFV status

17/40 (43%) subjects switched from an EFV-based combination. At baseline, some measurements appeared worse in individuals who did not switch from EFV. No significant difference was observed between groups in overall score changes at each time-point compared to baseline for all questionnaires except ISI, which, counter-intuitively, *improved* over 180 days in participants without efavirenz in their previous regimen and worsened in those with (p=0.02); this did not however remain after adjustment for multiple comparisons (p >0.05) (**appendix 1; table C**).

2.3.3.9. Relationship between sleep scores and PK parameters

There was no correlation between DTG PK parameters and D180 sleep scores or intra-subject change in global scores over 180 days (delta test scores) (**figure 2.2**; **appendix 2: tables D and E**). To rule out an effect dependent on a drug level threshold, the Mann-Whitney test was used to compare delta test scores in subjects with C_{max} above the upper quartile (Q4) to those below (Q1-3). There were no differences (0.62<p-value<1.0); nor with 95th centile C_{max} used as threshold (0.13<p-value<0.73). Similarly, there was no difference in C_{max} between D180 test score or delta test score low and high quartile groups for all sleep questionnaires (0.31≤p-value≤0.66 and 0.63<p-value<1.0).

2.3.4. Changes in cognitive scores

Between baseline and D180, no change in global cognitive composite scores and individual domain scores was observed over time except GML (executive function) where a significant improvement from baseline to D180 was seen (median change (IQR) 0.32 (0-0.74), unadjusted p=0.002).

There was no correlation between C_{24} and AUC_{0-24} and D180 cognitive function or delta cognitive scores (individual domains and global composite scores; p=0.07). Unexpectedly, higher C_{max} was associated with *improvements* in global cognitive function (r=0.39, p=0.02 **figure 2**). The improvement in median (IQR) delta score was higher in those with a C_{max} >upper 95% CI than in those below (p=0.0195) (**table 2.4**).

Cogstate domain	**Cognitive function**	**Standardised change score (Day 180-baseline)**		
		n	**Median (IQR)**	***p-value***
Detection task	Psychomotor function	37	0.02 (-0.16,0.13)	*0.743*
Identification task	Attention	37	-0.04 (-0.47,0.58)	*0.602*
Set Shifting	Executive function	37	0.05 (-0.32,0.75)	*0.471*
Groton Maze Learning	Executive function	34	0.32 (0.00,0.74)	*0.002***
Groton Maze Recall	Delayed recall	35	0.27 (-0.82,1.37)	*0.176*
One Card Learning	Learning	36	0.06 (-0.69,1.00)	*0.592*
One Back Memory	Working memory - simple	37	0.24 (-0.90,0.77)	*0.908*
Two Back Memory	Working memory - complex	37	0.00 (-0.97,0.84)	*0.982*
Composite score		37	0.16 (-0.23,0.37)	*0.187*
	C_{max} <95th centile (n = 25)	**C_{max} >95th centile (n=12)**		***p-value***
Median Cogstate Delta score (IQR)	0.08 (0.30-0.20)	0.41 (0.12-0.64)		0.0195*

Note: for difference scores, score sign reversed for all outcome measures where increasing values indicate performance decline. Thus, for all measures, negative values indicate performance decline and positive values indicate performance improvement. Difference scores standardised according to within-subject standard deviation (WSD). Composite score for each subject calculated by averaging standardised change scores across all domains. P-values are exact derived from Wilcoxon matched-pairs sign-rank test (not adjusted for multiple comparisons). *p <0.05, **p <0.01

Table 2.4: Change in neurocognitive scores (effect size)

2.3.5. Clinical safety and efficacy

2/43 (4.6%) participants discontinued the study secondary to AEs (described above). In the remaining subjects, there were no virological failures or grade 3 or 4 toxicity following treatment initiation. The studied FDC was well tolerated.

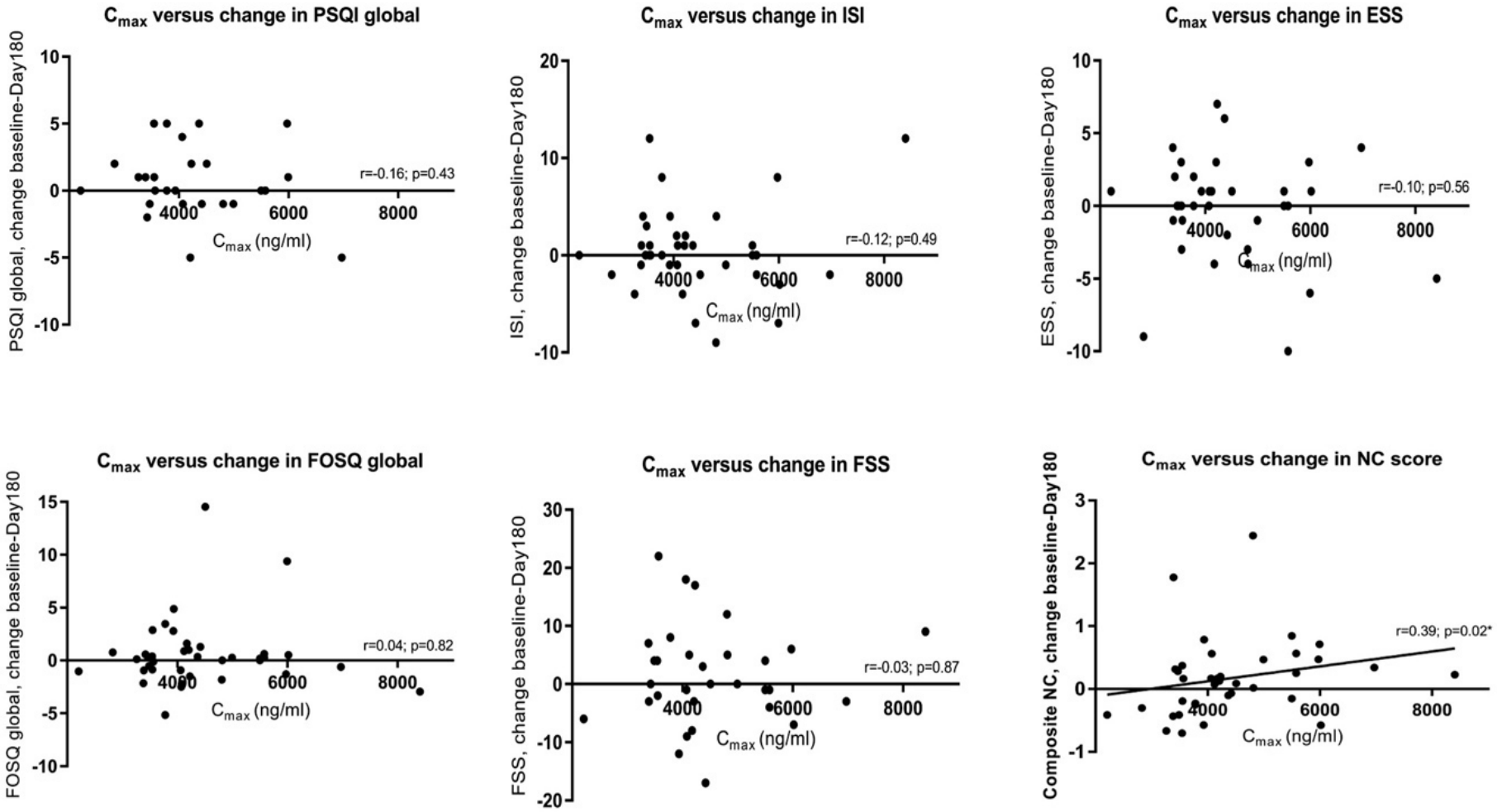


Figure 2.2: Scatter plots showing changes in sleep and neurocognitive scores in 180 days against C_{max}

2.4. DISCUSSION

The steady-state PK of DTG 50 mg OD was characterised in an aging HIV population, mostly over 65 years, the age associated with potential changes in drug PK.[245] Compared to the younger control group, C_{max} was significantly higher (25%) in those ≥60, indicating increased DTG absorption. Whilst the net effect of age-related physiological intestinal changes (e.g. reduction in pH, gastrointestinal motility etc.) on the absorption of most drugs is thought to be minimal,[239, 257] our findings could potentially be explained by age-related alterations in expression of active DTG efflux transporters, such P-gp and BCRP, across epithelial cells in the gastrointestinal tract;[239, 245, 258] however further research is required. There were no differences in DTG C_{24}, AUC_{0-24} or $t_{1/2}$ between the two groups, supporting a lack of age-associated effect on the main DTG metabolic pathway (UGT1A1).

To address the call for prospective PD data, this report describes the first post-marketing analysis of sleep and cognition-related PD changes following a switch to ABC/3TC/DTG, over 180 days.[125, 241] DTG-related NP-AEs (including insomnia) are an emerging concern and older age has been described as an independent risk factor. [125-151] In this study, two participants discontinued DTG because of NP-AEs (4.6%), which is consistent with published cohorts (1.4-7.2%). However, when investigating sleep quality and Cogstate status in those who continued the drug, we only observed a small increase in PSQI scores at D28, which resolved by D90, and a non-significant trend towards an increase in FSS score. Other scores remained stable or improved following the introduction of DTG. Whilst the one subject with PK measurements who withdrew secondary to NP-AEs had elevated levels of DTG, we did not find any association between DTG PK parameters and changes in sleep scores in the remaining

subjects over time, which is in keeping with observations from Riva and Hoffman.[259, 151] There were also no changes in sleep scores in subjects with very high drug concentrations in whom, surprisingly, cognition improved significantly. These interesting findings suggest that the mechanisms of DTG-related neurotoxicity are likely to be more complex than a simple linear or threshold-defined PK relationship and may relate to a combination of factors that include pharmacogenetic, immune and/or functional predispositions. Of interest and mentioned in the introduction, Yagura *et al.* and reported that DTG C_{24} (≥1.06 μg/mL) correlated with CNS side effects in younger Japanese PLWH. No significant difference in DTG concentration was, however, observed with individual symptoms or insomnia. The researchers subsequently reported a weak association with *UGT1A1**6 and *UGT1A1**28 alleles.[148]

Capetti *et al*. found DTG-related sleep disorders resolved in some patients switching to morning dosing (0.9% *vs* 3.5%).[134] In the study reported here, subjects were dosed in the morning to allow for steady state PK measurements; this could partially explain the absence of new sleep disturbances, although other researchers report unchanged rates of NP-AEs with morning dosing.[148, 241] The present subject population was a group of only mildly sleep-disturbed individuals from baseline, which may also partially explain the lack of positive findings in those who completed the study. Overall, whilst sleep impairment rates (PSQI >5) at baseline, matched that historically reported in the HIV literature (44-51%), scores were only just in the lower range of abnormal (≤7). Additionally, the prevalence of subjects with moderate insomnia (ISI) in this cohort (7-21%) is below that previously reported in PLWH.[242]

Controlling for a switch from EFV did not change the lack of positive results; this likely to be due to the fact that the EFV subjects in this study were stable on it and are therefore a self-selected group of patients who do not experience major sleep disturbances on EFV.

There are limitations to this study. Subjects were predominantly male, thereby not fully representative of real-life cohorts. DTG NP-AEs are thought to be higher in women, although it is an *independent* risk factor. Importantly, the study was not powered to detect changes in sleep quality but for the ability to detect PK differences between younger and older PLWH, PD results should therefore be interpreted with caution (although the numbers mirrored previous HIV sleep studies[242] and consistency across sleep tools (measuring the same effect) suggest that results are accurate). Furthermore, the use of self-reported questionnaires may compromise intra- and inter-subject consistency and lead to recall bias. The effect of suggestion may also introduce bias as was proposed by the authors of the SINGLE trial (EFV *vs* DTG)[69] to explain higher rates of DTG-related sleep disturbances. Although validated in the general population, the sleep questionnaires used in this study are not validated in aging PLWH. Nevertheless, a good correlation between direction changes reflects good inter-questionnaire reliability. Finally, the use of historical controls is a limitation, which should be addressed in future studies with a larger and active control arm.

The strengths of this study lie in its prospective and controlled design, investigating a special population in need of data, which is growing in size and requires appropriate HIV treatment tailoring. Additionally, it is the first to characterise detailed sleep and cognitive data in PLWH following the introduction of Triumeq® and to explore the

DTG PK/PD relationship in aging PLWH. The use of multiple questionnaires also allowed a more comprehensive evaluation of sleep and its effects than previously reported.

In conclusion, a significantly higher DTG C_{max} was seen in PLWH ≥60 *vs* younger subjects. The discontinuation rate was similar to previous real-life reports but the C_{max} increase was not associated with sleep or cognitive decline over six months. This data informs physicians and patients on the safety and tolerability of DTG in older patients, particularly following the early period where careful monitoring remains recommended.[138]

CHAPTER 3

Exposure of Dolutegravir and of Cobicistat-boosted Elvitegravir and Protease Inhibitors Following Cessation of Drug Intake

CITATIONS

Elliot E, Amara A, Jackson A, Moyle G, Else L, Khoo S, Back D, Owen A, Boffito M. Dolutegravir and elvitegravir plasma concentrations following cessation of drug intake. *Journal of Antimicrobial Chemotherapy*. 2016 Apr;71(4):1031-6.

Elliot ER, Amara A, Pagani N, Else L, Moyle G, Schoolmeesters A, Higgs C, Khoo S, Boffito M. Once-daily atazanavir/cobicistat and darunavir/cobicistat exposure over 72 h post-dose in plasma, urine and saliva: contribution to drug pharmacokinetic knowledge. *Journal of Antimicrobial Chemotherapy*. 2017 Jul 1;72(7):2035-2041.

CONTENT

3.1. INTRODUCTION

Despite advances in antiretroviral therapy (ART) facilitating better adherence to HIV treatment, delay or omitted doses still occur, potentially leading to drug underexposure, virological failure and selection of drug-resistance mutations, conditioning future choice of ART. Therefore, plasma PK data after cessation of ARV drugs are important to understand and guide the management of late and missed doses, particularly in drugs, such as the protease inhibitors and the integrase inhibitors, that are used in complex cases of viral resistance, poor adherence and extensive antiretroviral treatment experience.[56, 260, 261] Drug persistence in plasma is dependent on its half-life (which itself depends on clearance, CL, and volume of distribution, V).[262] ARV agents with longer half-life may be more forgiving and allow for forgotten doses, especially if drug concentrations remain above therapeutic concentrations until the patient reinitiates drug intake.

In addition to information on 'forgiveness', PK data after cessation of intake may also inform the appropriateness of specific compounds for HIV pre-exposure prophylaxis (PrEP) and for alternative treatment strategies tailored to facilitate adherence, such as described in the FOTO and BREATHER studies.[263] The former demonstrated that an ARV strategy that includes structured, short-cycle, treatment interruptions (dosing for 5 days consecutively followed by a 2-day break) with ART containing TDF/FTC/EFV, all long $t_{1/2}$ agents, was non-inferior in HIV-infected adults to continued daily therapy and resulted in no virological failures, whilst being preferred by patients.[263] The BREATHER study, carried out in both, LMIC and high-income countries (HIC), demonstrated similar efficacy of the same strategy in adolescents[264]

In vivo data for the InSTIs, DTG and EVG/COBI, concentration decay after intake cessation have not been previously described. Neither have they been investigated in the PIs, DRV and ATV, when they are boosted by COBI rather than RTV. This chapter describes two pharmacokinetic tail studies in HIV-negative healthy volunteers that aim to address these gaps in pharmacokinetic knowledge.

InSTIs are the newest ARV class approved and as described earlier, they are increasingly favoured over older drug classes thanks to their high efficacy and tolerability.[265] EVG, is prescribed in combination with the CYP3A4 inhibitor, COBI, which enhances EVG exposure and enables its once-daily dosing, whilst DTG does not require pharmacological boosting. Both are available in a FDC taken once-daily, thereby facilitating potential for adherence.[266-268]

Conversely, most PIs do require pharmacological boosting.[260] RTV-boosted PIs such as ATV and DRV have been used for many years and remain an instrumental option as third agents in the management of HIV. As discussed in **chapter 1**, advantages of pharmacological boosting include increased drug exposure and a prolonged half-life allowing OD dosing, and in the case of PIs, achieving a high genetic barrier to resistance.[260] COBI, the newer pharmacokinetic enhancer lends itself to co-formulation and lacks enzyme-inducing activity, thereby reducing pill burden and potentially offering a better drug interaction profile than RTV.[198, 199] At 150 mg OD, it provides bioequivalent exposures of ATV (300 mg OD) and DRV (800 mg OD) compared with those observed with 100 mg of RTV OD.[198, 199, 204, 211]

Previously published data on the pharmacokinetic forgiveness of OD RTV-boosted

DRV and ATV, showed a favorable ATV PK tail and a slight increase in decline rate for both protease inhibitors as RTV concentrations decrease.[269]

The objectives of the two studies described below were to independently evaluate the plasma PK of once-daily InSTIs, DTG and EVG/COBI, up to 9 days (216 hours) following cessation of drug intake and the plasma PK of once daily PIs, ATV and DRV, boosted by COBI up to 72 hours following cessation of intake, in healthy volunteers.

3.2. METHODS

3.2.1. Participants

Written informed consent was obtained from male and non-pregnant, non-lactating female healthy volunteers aged between 18 and 65 years old and with a BMI between 18-35 kg/m^2. Participants were excluded if they had any significant acute or chronic medical illness, abnormal physical examination, ECG or clinical laboratory determinations; positive screens for HIV, hepatitis B or C; current or recent (within three months) gastrointestinal disease; clinically relevant alcohol or drug use that the investigator felt would adversely affect compliance with trial procedures; exposure to any investigational drug or placebo within three months of the first dose of the study drug; use of any other drugs, including over the counter medications and herbal preparations, within two weeks before the first dose of the study drug; and previous allergy to any of the constituents of the pharmaceuticals administered during the trial.

3.2.2. Study design

Both studies were open-label, two-phase PK trials carried out at the Clinical Trial Unit

of the St. Stephen's Centre, Chelsea, and Westminster Hospital, London, United Kingdom. Participant involvement was 38 days in Study 1 (DTG and EVG/COBI) and 33 days in study 2 (ATV/COBI and DRV/COBI), excluding screening and follow up visits.

At screening, participants had a clinical assessment and routine laboratory investigations performed. The safety and tolerability of study medications were evaluated throughout the trial (on safety visit days, PK days and at follow-up) using the 2004 NIAID Division of AIDS table for charaterising and grading the severity of adult and paediatric AEs described previously.

3.2.2.1. Study 1 (InSTIs):

After successful screening, participants were administered DTG 50 mg (Tivicay®) OD for 10 days for the first phase of the study. They were admitted to the unit on D10. Blood samples for DTG PK assessment were taken before the final dose in the morning of D10 and at 2, 4, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 192- and 216-hours post-dose. After a washout period of nine days, on day 20, all subjects were administered fixed-dose combination TDF/FTC/EVG/COBI 245/200/150/150 mg (Stribild®) OD for 10 days for the second phase of the trial and blood samples were taken at the same intervals as above, prior to and over 216 hours following the final dose. On the PK days, study medication intake was witnessed and taken with a standardised breakfast (626 kcal) and 240 mL of water and subjects were admitted for 12 hours, after which they could leave the unit and return at specified intervals to complete sampling over nine days.

3.2.2.2. Study 2 (PI/COBI):

After successful screening, volunteers were administered fixed-dose combination

ATV/COBI 300/150 mg (Evotaz®) OD in the morning for 10 days. On D10, participants were admitted and blood samples for ATV and COBI PK assessment were taken pre-dose and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, and 72 hours post-dose. After a washout period of seven days, all subjects were administered fixed-dose combination DRV/COBI 800/150 mg (Rezolsta®) OD for 10 days. On study D30, sampling for DRV and COBI plasma PK was taken pre-dose and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, and 72 hours post-dose. On the PK days, study medication intake was witnessed and taken with a standardized breakfast (626 kcal) and 240 mL of water. Study staff assessed compliance with study drug administration using direct questioning and pill count, throughout both studies.

3.2.3. Ethics

The study protocol for study 1 was approved by the London Westminster Research Ethics Committee, London, United Kingdom whilst the study protocol for study 2 was approved by the Bloomsbury Research Ethics Committee, London, United Kingdom. Both studies were also approved by MHRA UK and were conducted according to Good Clinical Practice and the Declaration of Helsinki (Study 1: NCT02219217; Study 2: NCT02589158).

3.2.4. Plasma collections for DTG, EVG, COBI, ATV and DRV

Blood samples were collected into lithium heparin containing-blood tubes (6 mL) at each time-point, immediately inverted several times and then kept on ice or refrigerated until centrifugation. Within 30 minutes of blood collection, each blood sample was centrifuged for 10 minutes at 2000 g at 4°C. Plasma was then aliquoted equally into three 2.0 mL tubes (Sarstedt Germany) and stored at -20°C. Samples were shipped on dry ice to the Liverpool Bioanalytical Facility for analysis. The laboratory

is Good Clinical Laboratory Practice-accredited and participates in an external quality assurance scheme (KKGT, the Netherlands).[270, 271]

3.2.5. Quantification of plasma DTG, EVG, COBI, ATV and DRV

Quantifications of DTG, EVG, COBI, ATV and DRV in plasma were determined using liquid-liquid extraction (with methyl tertiary-butyl ether) of analyte and internal standard (d5-DTG, d6-EVG, quinoxaline, ATV-d5 and DRV-d9) using validated high-pressure liquid chromatography tandem mass spectrometry and analytical conditions described by the Liverpool University group in the literature.[272, 273]

The lower limit of quantification (LLQC) was 0.75 ng/mL for all plasma analyses in study 1, 10 ng/mL for ATV and 15 ng/mL for DRV in study 2. For concentrations below the assay limit of quantification, a value of one-half of the quantification limit was used in both studies.

In study 1, the assay was validated over a calibration range of 10-4000 ng/mL and 0.75-20 ng/ml (for concentrations below the LLQC of the initial assay). Accuracy (percentage bias) was between 98.0% and 104.6% (DTG), 101.8% and 106.7% (EVG), 99.8% and 106.2% (COBI) and precision was between 4.6% and 6.2% (DTG), 4.3% and 5.6% (EVG) and 5.0% and 6.0% (COBI).

In study 2, the assay was validated over a calibration range of 3.7-500 ng/mL for all three analytes. Accuracy (percentage bias) was between 99.5% and 108.2% (ATV), 94.2% and 101.2% (DRV) and 92.3% and 104.0% (COBI), and precision was between 2.8% and 5.4% (ATV), 4.4% and 6.0% (DRV) and 3.1% and 6.5% (COBI).

3.2.6. Pharmacokinetic and statistical analysis

The calculated PK parameters for DTG, EVG, COBI, ATV and DRV were the plasma C_{max}, AUC_{0-24} and C_{24}. The half-life was determined from the elimination phase within the normal dosing interval of 0 to 24 hours and as a terminal elimination half-life to the last measurable concentration within 216 hours for DTG and EVG/COBI and within 72hrs for ATV/COBI and DRV/COBI. All PK parameters were calculated using actual blood sampling time and non-compartmental modelling techniques (WinNonlin Phoenix, version 6.1; Pharsight Corp., Mountain View, CA).

Descriptive statistics, including GM and 90% or 95% CI were calculated for all 5-drug PK parameters. GMs were compared to the suggested therapeutic targets established in vitro (DTG, EVG and DRV) or in vivo (ATV), currently available in the literature. For the integrase inhibitors, this is the population protein binding-adjusted (PA) inhibitory concentration at 90% (IC_{90}) for wild type (WT) virus for DTG (64 ng/mL) and the PA-IC_{95} for EVG (45 ng/mL). For the PIs, the suggested therapeutic target is the consensus minimum trough concentration (equivalent to 10 times the protein-binding-corrected IC_{50} for WT virus), which is 150 ng/mL for ATV for wild type virus and 550 ng/mL for DRV for resistant virus, as defined at the 7th International Workshop on Clinical Pharmacology of HIV.[76, 274-276] Inter-individual variability in drug PK parameters was expressed as a percentage coefficient of variation [CV%].

3.3. RESULTS

3.3.1. Study populations

3.3.1.1. Study 1

Seventeen participants completed all phases of study 1. The median (range) age was 39 (26-52) years, and the median BMI (range) was 26 (19-34) kg/m^2. Twelve participants were female; nine described themselves as Caucasians and eight as Black.

3.3.1.2. Study 2

Sixteen volunteers completed all phases of study 2. Median (range) age and BMI were 38 (24-54) years and 25 (22-31) kg/m^2, respectively. Nine were female. Nine described themselves as Caucasian, six as Black and two as Asian.

3.3.2. Drug plasma pharmacokinetics

3.3.2.1. Study 1: DTG and EVG/COBI

PK parameters for DTG, EVG and COBI are summarised in **tables 3.1** and **3.2**. GM plasma concentration *vs* time curves are shown in **figure 3.1**.

Dolutegravir plasma pharmacokinetics

The GM terminal elimination half-life (90% CI) for DTG was 23.1 hours (19.7-26.6). This value was higher than the half-life measured over the dosing interval of 24 hours (14.3 hours; 12.9-15.7).

The PA-IC$_{90}$ for DTG is 64 ng/mL.[76] GM plasma DTG concentrations were measured above this value in all participants, at 24, 36, and 48 hours post-cessation of drug intake. At 60- and 72-hours post-drug intake cessation, 16 out of 17 subjects had DTG concentrations above the PA-IC$_{90}$. At 96 hours post-dose, DTG GM concentration fell below the PA-IC$_{90}$ (52.2ng/ml, range 6.9-153.0), with four subjects remaining above the PA-IC$_{90}$ (**table 3.1; figure 3.2**).

Elvitegravir plasma pharmacokinetics

EVG GM terminal elimination half-life (90% CI) to the last measurable concentration was 5.2 hours (4.7-6.1), which was lesser than the half-life measured over the dosing interval of 24 hours (10.8 hours, 9.7-13.0).

The suggested PA-IC_{95} for EVG is 45 ng/mL.[9] All subjects had EVG concentrations above the PA-IC_{95} at 24 hours post-dose. EVG GM plasma concentration was above the PA-IC_{95} 36 hours post-drug cessation (GM 57 ng/mL, range from 11 to 296 ng/mL), however only 11/17 subjects had EVG concentrations above it. The EVG GM concentration fell below the PA-IC_{95} at 48, 60- and 72-hours post-drug intake cessation and EVG concentrations were below the lower limit of quantification in all study participants at 96 hours post-dose (**table 3.1; figure 3.3**).

Cobicistat plasma pharmacokinetics when combined with EVG

The GM terminal elimination half-life (90% CI) to the last measurable concentration for COBI was 18.2 hours (16.2-26.0). This was higher than the half-life measured over the dosing interval of 24 hours (3.5 hours, 3.3-3.9) (**table 3.2; figure 3.1**).

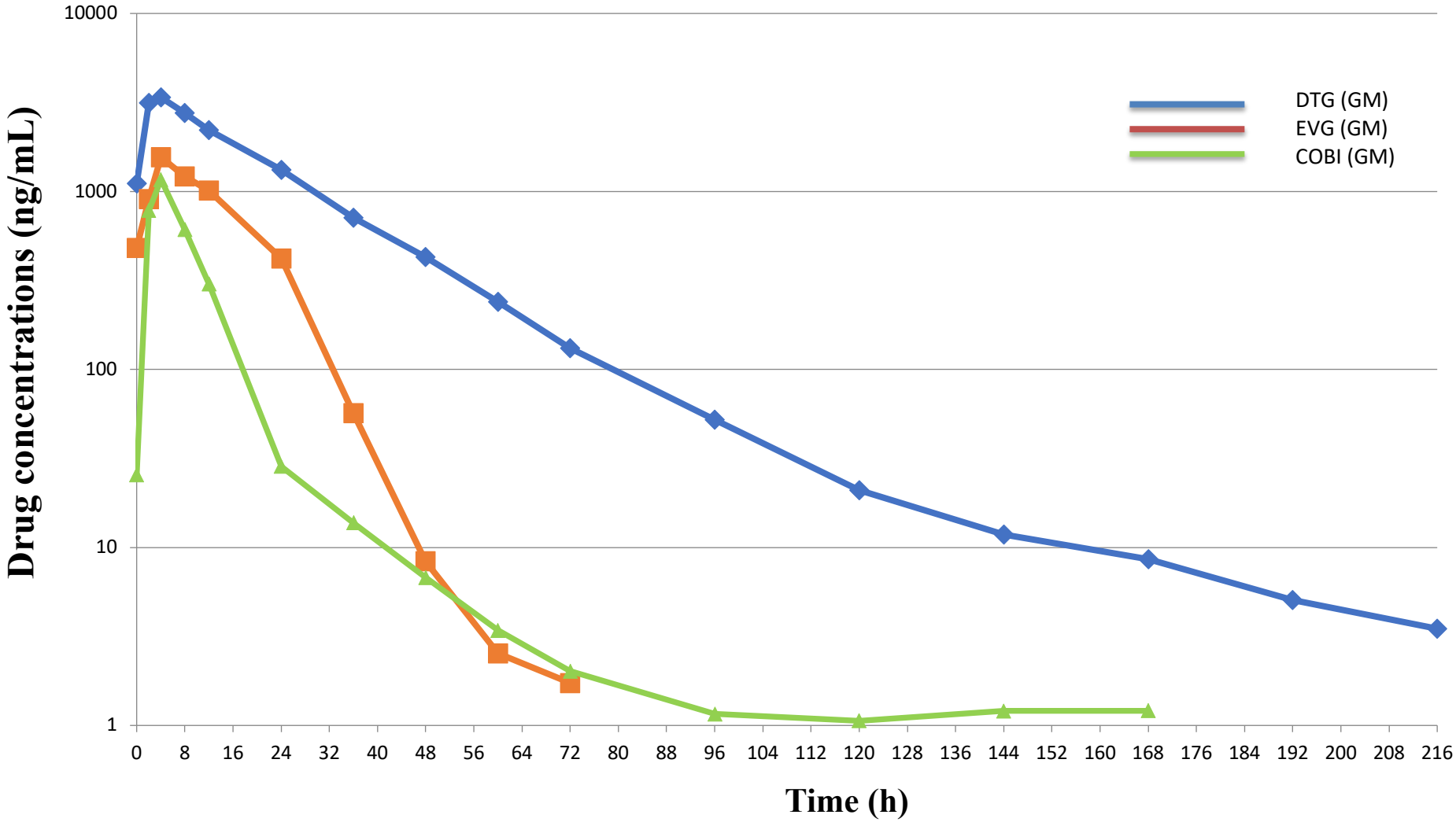


Figure 3.1: GM plasma concentration *vs* time curves for DTG, EVG and COBI

Hour post-dose	Variable	Dolutegravir (PA-IC_{90} 64 ng/mL)	Elvitegravir (PA-IC_{95} 45 ng/mL)
24hrs	GM concentration (ng/mL)	1324	419
	Proportion detectable in plasma	100% (17/17)	100% (17/17)
	Proportion above IC_{90} or IC_{95}	100% (17/17)	100% (17/17)
36hrs	GM concentration (ng/mL)	711	57
	Proportion detectable in plasma	100% (17/17)	100% (17/17)
	Proportion above IC_{90} or IC_{95}	100% (17/17)	65% (11/17)
48hrs	GM concentration (ng/mL)	427	8.3
	Proportion detectable in plasma	100% (17/17)	94% (16/17)
	Proportion above IC_{90} or IC_{95}	100% (17/17)	0%
60hrs	GM concentration (ng/mL)	240	2.5
	Proportion detectable in plasma	100% (17/17)	76% (13/17)
	Proportion above IC_{90} or IC_{95}	94% (16/17)	0%
72hrs	GM concentration (ng/mL)	131	1.7
	Proportion detectable in plasma	100% (17/17)	53% (9/17)
	Proportion above IC_{90} or IC_{95}	94% (16/17)	0%
96hrs	GM concentration (ng/mL)	52.2	—
	Proportion detectable in plasma	100% (17/17)	0%
	Proportion above IC_{90} or IC_{95}	23.5% (4/17)	—

Table 3.1: Summary of dolutegravir and elvitegravir concentrations (expressed as GM) and detectability at significant time points

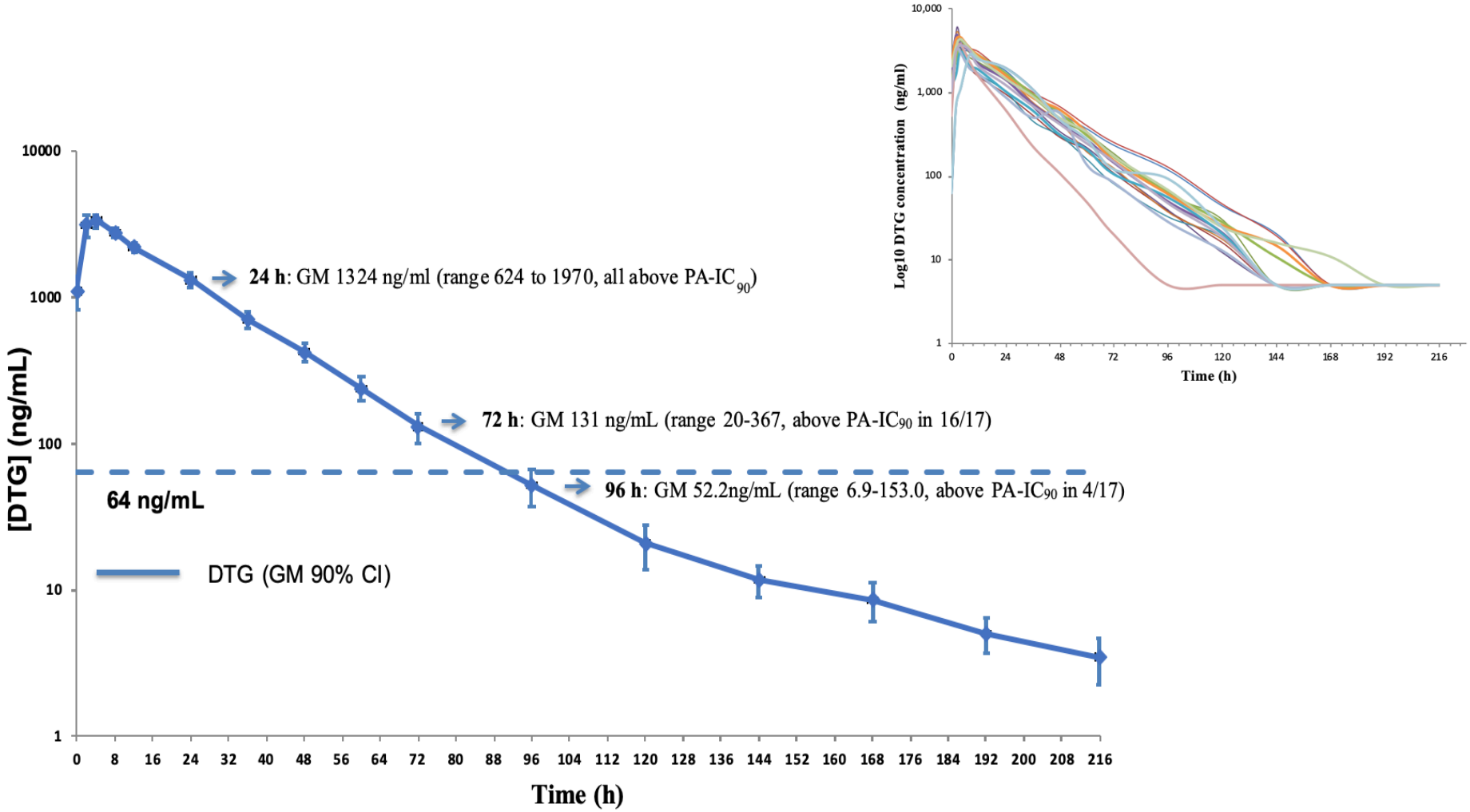


Figure 3.2: DTG Plasma concentrations over time and proportion of subjects with DTG concentration above PA-IC$_{90}$ at key time points. Top right corner: concentration-time curve for each individual subject showing spread of the concentration decay data

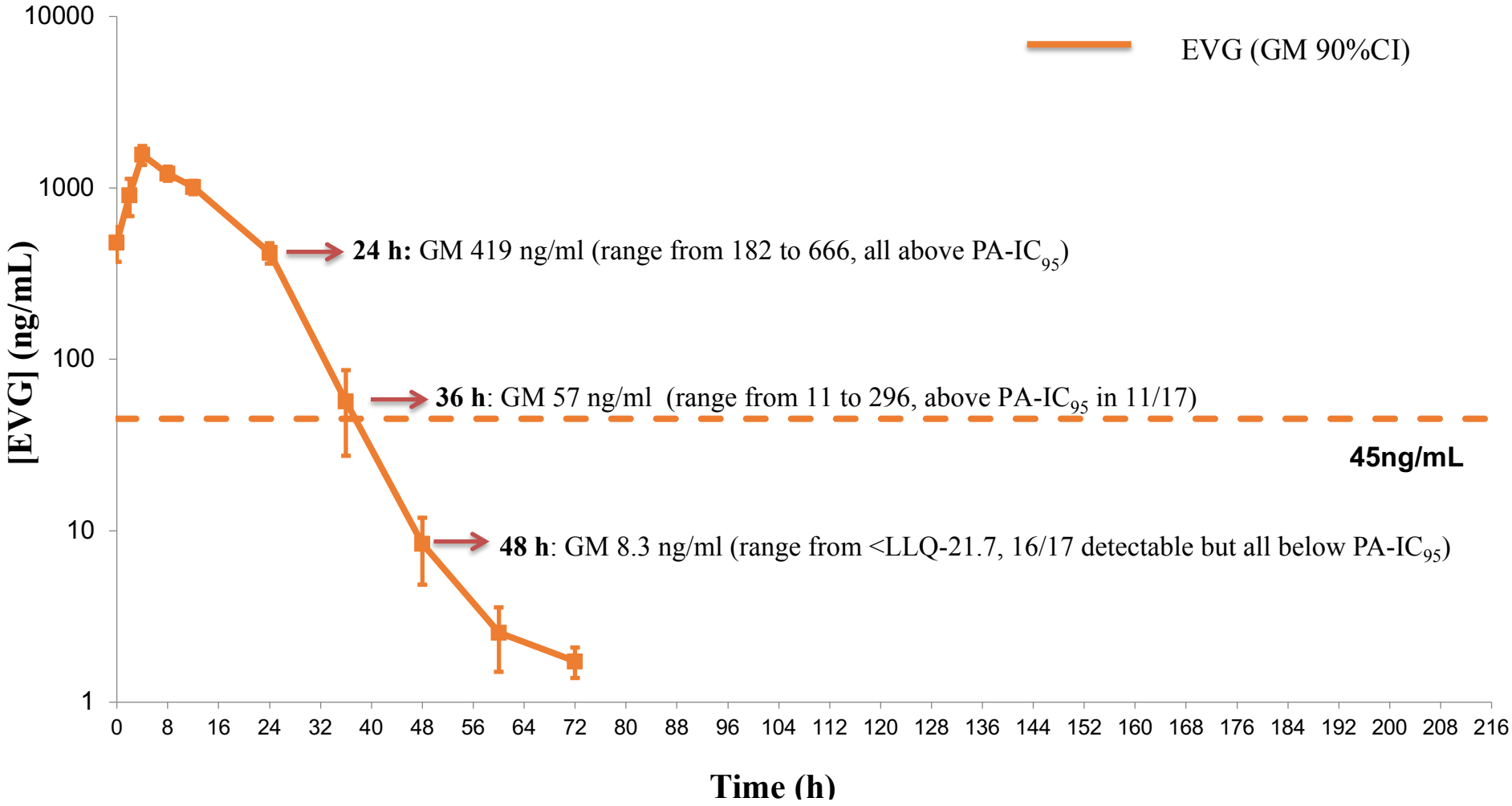


Figure 3.3: EVG Plasma concentrations over time and proportion of subjects with DTG concentration above PA-IC$_{90}$ at key time points

PK parameters	DTG		EVG		COBI	
	GM (90% CI)	CV %	GM (90% CI)	CV %	GM (90% CI)	CV %
t_{Max} (hours)	3.1 (2.4-3.9)	55	4.5 (4.1-4.6)	39	3.1 (2.9-3.7)	30
C_{max} (ng/mL)	3908 (3571-4245)	21	1675 (1557-1884)	24	127 (1184-1437)	24
AUC_{0-24} (ng.h/mL)	55505 (51368-59642)	18	22965 (21483-25592)	22	10686 (9692-12522)	32
C_{24} (ng/mL)	1324 (1178-1470)	27	419 (387-501)	32	28 (24-48)	85
C_{48} (ng/mL)	427 (362-499)	35	8 (8-15)	78	7 (6-9)	47
$t_{1/2}$ (0-24) (hrs)	14.3 (12.9-15.7)	23	10.8 (9.7-13.0)	31	3.54 (3.3-3.9)	20
$t_{1/2}$ (last) (hrs)	23.1 (19.7-26.6)	16	5.2 (4.7-6.1)	18	18.2 (16.2-26.0)	57

C_{48}: concentration 48 hours post-dose

Table 3.2: Plasma dolutegravir and elvitegravir/cobicistat PK parameters

3.3.2.2. Study 2: ATV/COBI and DRV/COBI

ATV and DRV PK parameters are summarised in **table 3.3**.

Atazanavir plasma pharmacokinetics

ATV GM plasma concentration *vs* time curves when combined with COBI are shown in **figure 3.4**. The GM terminal elimination half-life to 72 hours of ATV was 6.77 hours (95% CI 6.2-7.5). This value was lower than the half-life measured over the dosing interval of 24 hours (GM 9.69 hours; 95% CI 9.2-12.8).

All subjects had ATV concentrations above the suggested target 24 hours post-dose

(GM [range] 759.2 [249-2667] ng/mL). Out of 16 subjects, 2 and 13 had concentrations below the target at 30 and 48 h post-dose, respectively (GM 407.0 and 65.9 ng/mL, **table 3.4**). The inter-individual variability in ATV C_{24} was 73%.

Darunavir plasma pharmacokinetics

Darunavir GM plasma concentration *vs* time curves when combined with COBI are shown in **figure 3.4**. DRV GM terminal elimination half-life was 6.4 hours (95% CI 5.9-7.0). This value was lower than the half-life measured over the dosing interval of 24 hours (GM 10.4h; 95% CI 9.2-12.9).

Out of 16 subjects, 3 had DRV concentrations below the suggested target 24 hours post-dose and 11 had concentrations lower than the target at 30 hours (GM [range] 1032.6 [373-3359] and 381.7 [97-2057] ng/mL respectively; **table 3.4).** Of note, the GM DRV concentration was 1202 ng/mL, at 20 hours, the last sampling time before 24 hours. The inter-individual variability in DRV C_{24} values was 65%.

Cobicistat plasma pharmacokinetics

Steady-state COBI PK parameters when combined with ATV and DRV are reported in **table 3.5**. When combined with ATV, the GM terminal elimination half-life to the last measurable concentration for COBI was 4.2 hours (95% CI 3.9-4.7) and over the dosing interval of 24 hours was 4.4 hours, 95% CI 4.0–5.2).These were higher than when COBI was combined with EVG and with DRV.

When combined with the latter, GM terminal elimination half-life to the last measurable concentration of COBI was 3.6 hours (95% CI 3.3-4.0) and was 3.8 (95% CI 3.5-4.3) over the dosing interval of 24 hours.

PK parameters ATV 300mg OD

	$t_{1/2}$ (0-24h)	$t_{1/2}$ (0-C_{last})	C_{max} (ng/mL)	C_{24} (ng/mL)	C_{last} (ng/mL)	AUC_{0-24} (ng.h/mL)	$AUC_{0-Clast}$ (ng.h/mL)
GM	9.69	6.77	3718.85	759.20	6.36	37713	46129
low 95%	9.24	6.22	3308.00	612.57	1.29	32661	38592
up 95%	12.83	7.54	4940.55	1290.07	19.00	51556	67844
Min	6.32	5.42	844.97	256.10	5.00	11414	14058
Max	19.26	9.96	7282.82	2666.54	77.28	83763	128323
CV (%)	33	20	40	73	178	46	56

PK parameters DRV 800mg OD

	$t_{1/2}$ (0-24h)	$t_{1/2}$ (0-C_{last})	C_{max} (ng/mL)	C_{24} (ng/mL)	C_{last} (ng/mL)	AUC_{0-24} (ng.h/mL)	$AUC_{0-Clast}$ (ng.h/mL)
GM	10.41	6.35	5515.02	1032.56	8.80	58100	66710
low 95%	9.18	5.88	4949.07	837.92	6.01	51464	58145
up 95%	12.94	7.03	6566.03	1625.74	14.44	70391	83214
Min	5.23	4.25	2855.55	372.96	7.50	26404	29317
Max	19.15	8.48	8365.97	3359.34	41.13	111312	141982
CV (%)	35	18	29	65	84	32	36

Table 3.3: Plasma atazanavir (ATV) and darunavir (DRV) steady state pharmacokinetic (PK) parameters, expressed as geometric mean (GM) and 95% confidence intervals (CI), range (minimum, Min; maximum, Max) and coefficient of variation (CV), over 24 and 72 hours)

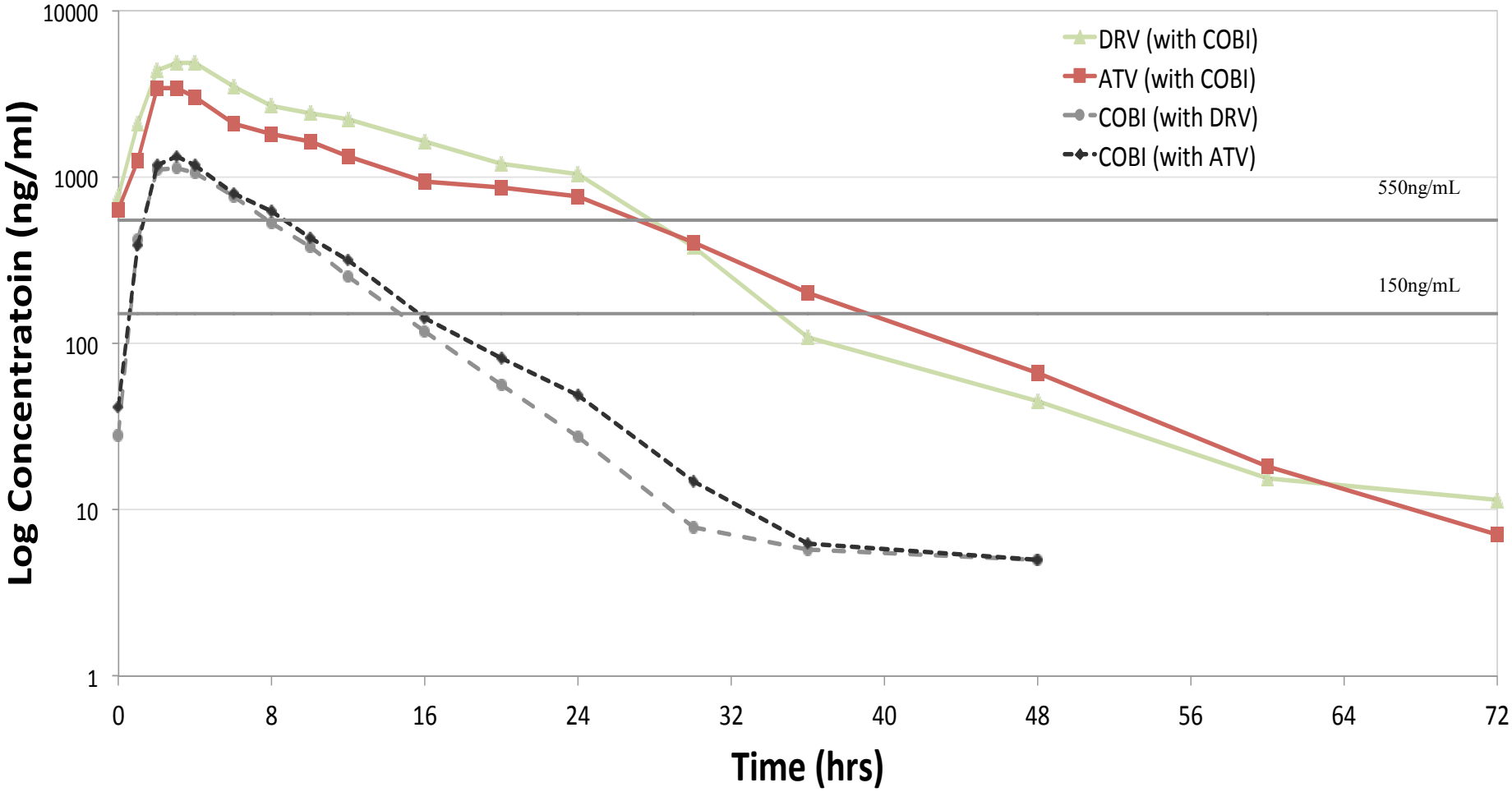


Figure 3.4: Plasma concentration *vs* time curves of atazanavir (ATV) and darunavir (DRV) when boosted by 150 mg of cobicistat (COBI) over 72 hours.

	Hours post-dose			
	24	30	36	48
ATV GM (range; ng/mL)	759 (249-2667)	407 (148-1679)	201 (65-1093)	66 (13.5-949)
No of subjects below target	0/16	2/16	5/16	13/16
DRV GM (range; ng/mL)	1033 (373-3359)	382 (97-2057)	109 (LLQ-594)	45 (LLQ-196)
No of subjects below target	3/16	11/16	15/16	16/16

Table 3.4: Plasma concentrations of atazanavir (ATV) and darunavir (DRV) measured at 24, 30, 36, 48 hours post-dose, expressed as geometric mean (GM) and range, and number (No) of subjects below target per time-point.

3.3.3. Safety and tolerability

No serious breaches to the protocols were recorded in either study. Study drugs in study 1 were well tolerated and no grade 3 or 4 adverse events were reported.

During study 2, treatment was generally well tolerated, and no serious adverse events occurred during the study. As expected because extensively described in the literature,[277] the most common adverse events observed throughout the study were scleral icterus and hyperbilibirubinaemia (during the ATV/COBI phase). No other clinically relevant changes in laboratory parameters were reporte

PK parameters COBI 150mg combined with atazanavir

	$t_{1/2}$ (0-24h)	$t_{1/2}$ (0-C_{last})	C_{max} (ng/mL)	C_{24} (ng/mL)	C_{last} (ng/mL)	AUC_{0-24} (ng.h/mL)	$AUC_{0-Clast}$ (ng.h/mL)
GM	4.43	4.21	1408.02	49.59	5.00	10554	10924
low 95%	3.95	3.87	1293.37	42.07	5.00	9589	9905
up 95%	5.19	4.69	1577.76	79.63	5.00	12059	12535
Min	3.14	3.21	929.72	14.15	5.00	7826	8145
Max	8.39	6.13	1986.37	156.24	5.00	14681	15068
CV (%)	28	19	20	63	0	23	24

PK parameters COBI 150mg combined with darunavir

	$t_{1/2}$ (0-24h)	$t_{1/2}$ (0-C_{last})	C_{max} (ng/mL)	C_{24} (ng/mL)	C_{last} (ng/mL)	AUC_{0-24} (ng.h/mL)	$AUC_{0-Clast}$ (ng.h/mL)
GM	3.81	3.62	1250.25	27.56	5.00	9532	9681
low 95%	3.49	3.34	1149.77	22.29	5.00	8678	8791
up 95%	4.29	3.98	1392.73	51.37	5.00	10857	11079
Min	2.59	2.59	932.46	5.00	5.00	6167	6254
Max	5.60	5.55	1867.32	120.90	5.00	14426	14933
CV (%)	21	18	20	81	0	23	23

Table 3.5: Plasma cobicistat (COBI) steady state pharmacokinetic (PK) when combined with atazanavir and with darunavir, expressed as geometric mean (GM) and 95% confidence intervals (CI), range (minimum, Min and maximum, Max) and coefficient of variation (CV), measured over 24 and 72 hours.

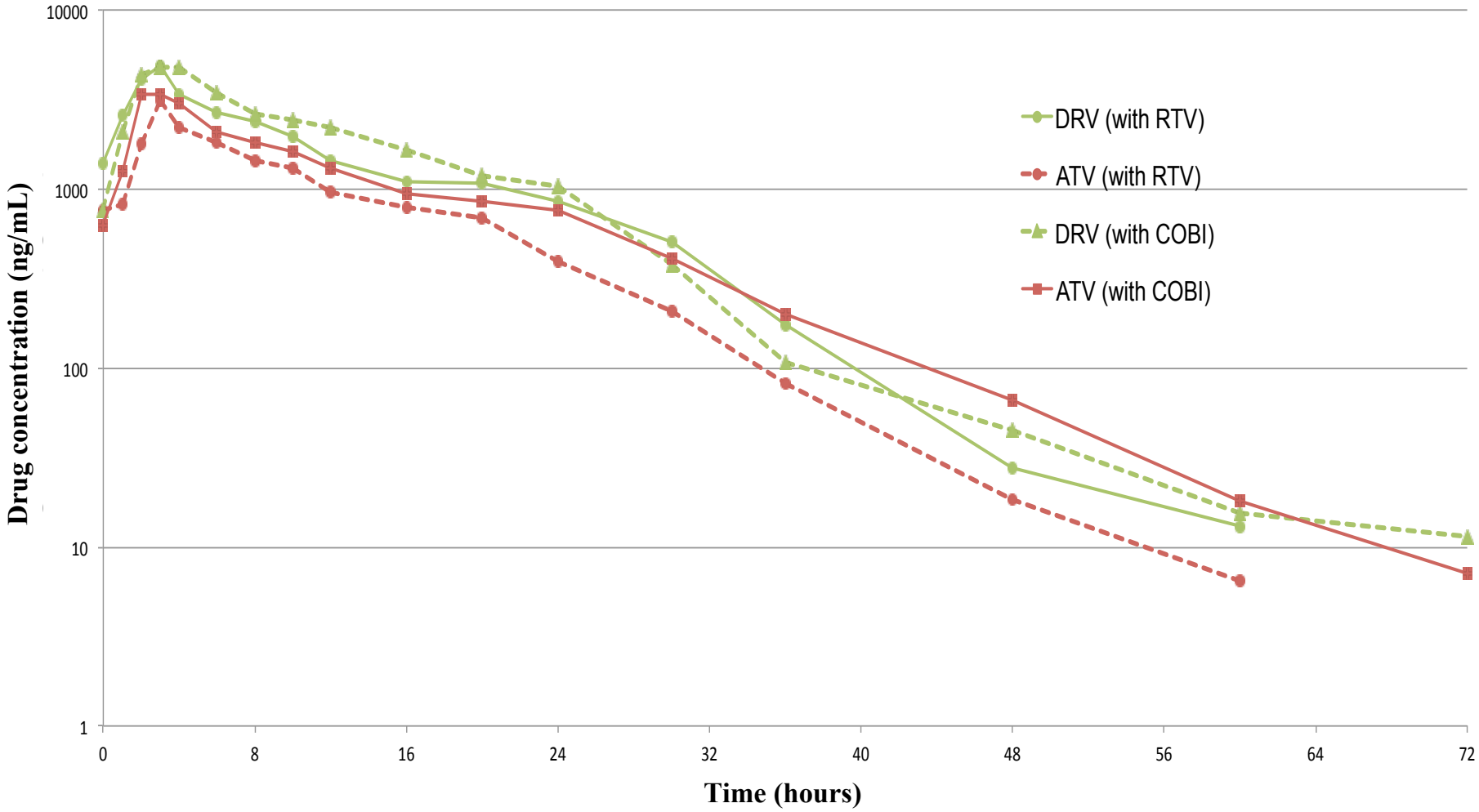


Figure 3.5: Atazanavir (ATV) and Darunavir (DRV) concentrations *vs* time curves when combined with cobicistat (COBI) compared with ritonavir (RTV) (Boffito *et al.* 2011)

3.4. DISCUSSION

In the first study, the steady-state plasma pharmacokinetics of DTG 50 mg OD and EVG 150 mg OD boosted by COBI (150 mg) over 10 days following drug intake cessation was reported, in 17 male and female healthy volunteers. This data fully characterised, for the first time, the PK forgiveness of the two newest InSTIs (at the time of publishing). Following achievement of steady-state, GM DTG concentrations remained above the suggested plasma PA-IC_{90} of 64 ng/mL for up to 72 hours post-drug intake cessation, with most subjects (94%) showing concentrations above the PA-IC_{90} at this time. The GM concentration for EVG was above the suggested PA-IC_{95} of 45 ng/mL at 24- and 36-hours post-drug cessation, with 65% of participants above this cut-off at the latter time point, but it had fallen below the PA-IC_{95} by 48 hours post-dose (with no participant above this cut-off then). At the time of the study, there were no established minimum effective therapeutic concentrations for either agents. Being above the (partially PA) *in-vitro* IC_{90} or IC_{95} does not imply that exposure is sufficient for a fully effective in vivo drug exposure, especially when optimal drug exposures are needed during induction of virological control. More recently, an *in-vivo* minimum effective concentration (MEC) of 324 ng/mL has been suggested for DTG, based on data from the initial 10-day monotherapy study, which showed that, with a Hill Factor of 1, it was associated with 90% of E_{max}.[57, 59] If this MEC is used, GM DTG C_{min} remained therapeutic for over 48 hours in our study, falling below target at 60 hours. Plasma inter-individual variability (CV) was 27% in DTG C_{24} and 32% in EVG $C_{24,}$ which is consistent with previously published data.[57] These values are considerably lower than those published for the third available InSTI, raltegravir (53%–220%).[278]

Interestingly, whilst DTG is not dependent on a booster and concentrations are persistent in the systemic circulation for a prolonged period; concentrations of EVG were observed to drop when the concentrations of its booster, COBI, fell below a certain level. The terminal $t_{1/2}$ (0 to 216 h) was longer than the $t_{1/2}$ within the dosing interval (0–24 h) for DTG, whilst the opposite was true for EVG (23.1 *vs* 14.3 hours and 5.2 *vs* 10.8 hours, respectively). One explanation for this phenomenon could be saturation of metabolic processes at higher concentrations, meaning a change in rate of CL as COBI concentrations fall to non-saturating levels.

Of note, the single-tablet formulations of DTG and EVG contain partner NRTIs of varying $t_{1/2}$ values. It has been previously shown that the plasma $t_{1/2}$ of ABC and 3TC are 3–4 hours and 5.7 hours, respectively, with intracellular half-lives of the active triphosphorylated metabolite of ABC (carbovir) and the active triphosphorylated metabolite of 3TC being 14.1 hours and 19 hours, respectively.[279] Exposures do differ between male and female subjects. The longer $t_{1/2}$ of FTC and TDF, both in plasma and peripheral blood mononuclear cell (PBMC; 31 hours and 37 hours and 164 hours and 39 hours, respectively)[280-283] may also be important to the clinical forgiveness of these regimens and the specific resistance mutations that are observed at failure. DTG is currently available in co-formulation with ABC and 3TC; it is interesting to note that the $t_{1/2}$ of carbovir and the active triphosphorylated metabolite of 3TC match DTG's $t_{1/2}$ both within the dosing interval (14 hours) and to the last measurable concentration (23 hours).

One of the limitations of study 1 is that DTG was administered alone, whilst EVG/COBI were co-administered as part of a single tablet combination therapy with

TDF/FTC. DTG administered within an ABC/3TC FDC was not considered justified, based on the risk of ABC hypersensitivity in HIV-negative subjects [284] and the known lack of effect of backbone NRTIs on the pharmacokinetics of either EVG or DTG. Additionally, it is not impossible that a minority of patients had residual exposure to low-dose DTG at the start of the EVG phase of the study, but this exposure is likely to be minimal, as PK sampling for EVG was carried out 19 days after the last dose of DTG and, importantly, DTG has no known impact on EVG/COBI metabolic pathways.

The second study reports the steady-state PK of COBI-boosted ATV 300 mg and DRV 800 mg in plasma over 72h following drug intake cessation in HIV-negative healthy volunteers, to describe the PK forgiveness of these two commonly used PIs when boosted by COBI (150mg). Concentrations of ATV were measurable in all subjects 48 hours post-dose and in 11 and 2 subjects 60- and 72-hours post-dose. Importantly 14/16 subjects had concentrations above the suggested minimum effective concentration (MEC) of 150 ng/mL and the remaining two had concentrations close to the MEC (148 ng/mL) 30 hours post-dose, suggesting that a 6-hour drug intake delay would not compromise optimal drug exposure and efficacy. Similarly, DRV concentrations were measurable in 13/16, 6/16 and 2/16 subjects 48, 60- and 72-hours post-dose, respectively. However, 3/16 study individuals had concentrations below the suggested 550 ng/mL cut-off 24 hours post-dose and only 5 had concentrations above 550 ng/mL 30 hours post-dose. Whether this is clinically significant is unclear and more data in patients who are poorly adherent to DRV/COBI are needed in the near future to help clinicians with prescribing the optimal booster in certain complex clinical situations (e.g. suboptimal viral replication suppression).

Notably, measurements of ATV PK forgiveness in the presence of COBI were similar to those in the presence of RTV where ATV terminal elimination half-life was 6.77 hours with COBI *vs* 6.74 hours with RTV (**figure 3.5**).[269] DRV terminal elimination half-life was measured at 6.35 hours with COBI *vs* 6.48 hours with RTV (**figure 3.5**). While there is no doubt of PI robustness in ARV-naïve PLWH, in patients who are inclined to poor compliance or harbour viral resistance, PK forgiveness knowledge may be particularly important.

In addition, we did not see the small increase in DRV plasma concentration at the end of the dosing period (C_{24}) relative to previous time points (i.e.C_{20}) described with both RTV and, to a lesser extent, COBI in bioequivalence studies.[204, 285] This effect remains unexplained; it has been tentatively attributed to either enterohepatic recycling or redistribution of cellular DRV into plasma as the effect of RTV or COBI on cellular influx or efflux transporters diminishes with dropping concentrations.[204] This effect is, however, unconfirmed and would require further investigation.

Both COBI and RTV inhibit CYP3A4, thereby reducing the metabolism of concomitantly administered PIs and leading to enhanced drug exposure.[198] Although very similar, the two drugs are not identical and their relationship with the therapeutic agent they enhance may explain concentration decay patterns. Importantly, the rates of decline of both ATV and DRV slightly increased as COBI concentrations declined. COBI itself is metabolised by CYP3A4 and when given with ATV, a moderate CYP3A4 inhibitor,[189] it achieves slightly higher concentrations than when co-administered with DRV, which, although also a CYP3A4 inhibitor, may have a lesser

effect.[189] COBI terminal half-life was 4.21 hours with ATV and 3.62 hours with DRV, therefore shorter than RTV terminal half-life with ATV (5.03 hours) and DRV (6.30 hours), respectively. The inter-individual variability (CV) in ATV and DRV C_{24} was 73% and 65% with COBI, therefore similar to those previously measured with RTV (81% and 62%, respectively).[269]

The cut-off values used in study 2 were those used in the published PK tail study for ATV and DRV when boosted with RTV, to allow for direct comparison between the studies.[269] They were the ATV MEC (150 ng/ml, 10-fold the in-vitro PA-IC_{50} calculated during drug development) and the DRV PA-EC_{50} for protease inhibitor-resistant strains (550 ng/ml) used as a reference by TDM services.[276] Of note, for treatment-naïve patients with wild-type virus, the DRV target is a lower 200 ng/ml.[286, 287]

There are limitations common to both studies. They were carried out in healthy volunteers so as not to impose ARV dose delays in patients infected with HIV. As such, PK/PD deductions or predictions on the *in-vivo* concentrations needed to maintain efficacy cannot be robustly drawn. Ideally, pharmacodynamics data in HIV infected participants are required to draw definite conclusions on how late a drug dose can be or how many drug doses can be missed before efficacy is lost.

Additionally, discrepancies in ARV drug pharmacokinetics between healthy volunteers and people living with HIV have been previously described, particularly for the PIs.[288] Such differences are thought to be related to physiological variability in several parameters between the two populations, including CYP450 activity and α-1-acid glycoprotein expression, and must be kept into consideration when interpreting data from healthy volunteers.[288] With regards to the InSTIs, intensive PK data from

licensing studies suggest that DTG and EVG concentrations may be moderately lower in healthy compared to HIV-infected subjects, but this is not thought to be significant.[57] Drugs in both studies were well tolerated, with adverse events limited to expected increases in indirect bilirubin levels during the ATV/COBI study phase.

PK forgiveness data is important, particularly in diseases that are chronic, where sub-optimal compliance to medications is common.[289] In the context of HIV infection, this is especially salient since therapy is life-long and insufficient drug exposure from missed doses can lead to the emergence of drug-resistant HIV strains and limit future therapeutic options. Great efforts are made to support and encourage patients with respect to the importance of adherence, but it is often unclear how delayed a dose can be or how many doses can be omitted before efficacy is lost. Additionally, and as previously mentioned, understanding the PK attributes of a drug and, more specifically, its PK forgiveness can also allow identification of potential candidates for PreP and alternative treatment strategies where optimal dosing frequency needs to be characterised. These studies on PK forgiveness address some of these issues and gaps in knowledge for commonly used InSTIs and PIs.

In conclusion, these data contribute to understanding whether doses, for the specific drugs investigated, can be delayed or missed and, if so, to what extent. In particular, marked differences were found in the elimination rates of DTG and EVG following treatment interruption. This suggests that clinical differences may emerge in patients who have suboptimal adherence. Although, the net risk or benefit of these elimination characteristics will depend upon all the components of the regimen taken.

CHAPTER 4

Pharmacokinetics Of Dolutegravir With And Without Darunavir/Cobicistat In Healthy Volunteers

CITATION

Elliot ER, Cerrone M, Else L, Amara A, Bisdomini E, Khoo S, Owen A, Boffito M. Pharmacokinetics of dolutegravir with and without darunavir/cobicistat in healthy volunteers. J Antimicrob Chemother. 2019 Jan 1;74(1):149-156. doi: 10.1093/jac/dky384.

CONTENT

4.1. INTRODUCTION

As discussed in the introduction, triple-drug therapy has been the cornerstone of HIV treatment since 1996, leading to unprecedented success in disease control in PLWH. With increasingly potent agents, there has, in recent years, been a drive to investigate treatment simplification strategies that aim to lessen toxicity, drug interactions and cost through reducing the number of drugs taken.[100] This is particularly salient since the HIV-infected population is aging and, with increasing comorbidities, polypharmacy is rising.[290] Current available evidence favours dual therapy over monotherapy and is most reassuring in suppressed patients who have maintained virological control for at least six months on triple therapy.[103]

DTG and boosted DRV (DRV/b) are both strong players in this paradigm shift and both have featured independently in most of the recent dual combinations investigated.[103] They are the agents with the highest potency and resistance barrier within their respective classes and overall,[291, 292] meaning that they are also important in salvage therapy in patients experiencing treatment failure and harbouring multi-class drug resistances (BD dosing recommended).[72, 293] They have both been paired individually with 3TC in dual therapy studies, with promising data in treatment naïve and in virologically suppressed patients; this is outlined in **chapter 1**.[106-109, 294-296] DTG combined with RPV has also been studied as maintenance therapy in two large RCTs (**SWORD 1&2**) and in smaller cohort studies, showing high efficacy, improved safety and cost savings, when used as maintenance therapy (albeit with slightly higher discontinuation rates secondary to AEs, 3% *vs* <1%).[112-116, 297] Of note, Diaz *et al.* reported that switching suppressed HIV-infected patients with multiple previous

treatment failures to standard dose DTG/RPV OD was effective through to 48 weeks, with improved safety profiles.[117]

However, the aforementioned options are not appropriate in the context of NRTI-related long-term toxicities and NRTI/NNRTI-associated resistance mutations.[102] In this setting, combining a PI and an InSTI offers an appealing NRTI-sparing alternative.[101, 102] The **NEAT001/ANRS143** study found DRV/RTV combined with RAL non-inferior to DRV/RTV/TDF/FTC based on clinical and/or virological failure at 96 weeks in 800 ART naïve individuals. However, the genetic barrier of the two-drug regimen in this context was lower than the three-drug regimen, with more frequent emergence of resistance in cases of VF, particularly in those with high baseline VL.[298, 299] Additionally, the **SPARE** pilot study in already virologically suppressed patients, suggested that RAL/DRV/RTV was effective in maintaining viral suppression.[300] This combination requires twice daily drug intake, whereas combining boosted DRV with DTG offers the benefits OD dosing and a high genetic barrier to resistance, since both agents have a high affinity for their target enzymes.[83, 301, 302]

A small number of cohort studies have been published on the use of DTG/DRV/RTV in multi-treatment experienced patients.[119-121]In Canada, Wheeler et al demonstrated high tolerability and maintenance of viral suppression after a mean of 12.8 (range 1-22) months in all of 13 HIV patients with primary transmitted thymidine analogue mutation (TAM) resistance, who switched from a complex salvage multi-drug regimen to DTG/DRV/RTV.[119] Similarly, in Italy, Capetti *et al.* followed 130 patients, with a current or past history of VF and documented viral resistance to one to five ARVs, who switched to DTG/DRV/RTV for simplification or rescue therapy. At 48 weeks, subjects with viral suppression increased from 60% to 94% and the metabolic impact

was favorable.[120] A single-point pharmacokinetic (PK) analysis in a subgroup of this study (32 subjects) confirmed adequate median C_{24} for both drugs (DTG 579 ng/mL; DRV 3007 ng/mL); the thresholds used were the in vitro PA-IC_{90} for wild type HIV for DTG (64 ng/mL) and the PA-IC_{50} for PI-resistant viruses, for DRV (550ng/mL).[274, 286] Five subjects were taking darunavir 600 mg BD and three were taking DTG BD (the majority of InSTI single mutations cause <10 fold changes in DTG sensitivity, BD dosing is recommended, however, in the presence of any InSTI resistance associated mutation patterns).[60, 76, 303] The **DUALIS** study, a recent large, prospective, interventional RCT (n=320) showed that OD DTG/DRV/RTV maintenance therapy in suppressed patient, was non-inferior to continuing DRV-based triple therapy, with high rates of maintained VLs and comparable rates of AEs demonstrated.[118] An intensive PK sub-study over 12 hours was published and described steady-state PK parameters for both drugs during co-administration (median C_{max} was 3427 ng/mL for DTG and 6170 ng/mL for DRV, C_{12} was 637 ng/mL for DTG and 1245 ng/mL for DRV and AUC_{0-12} was 26809 ng*h/mL for DTG and 49920 ng*h/mL for DRV), C_{24} was not measured.[304]

COBI may be preferable to RTV in some patients, in view of its lower potential for drug interactions than RTV and lower pill burden when co-administered.[198-200] However, PK data for DTG co-administration with DRV/COBI are very limited. DTG C_{24} doubled, when measured at least 10 days after switching DRV/RTV to DRV/COBI in a therapeutic drug monitoring (TDM) survey of HIV infected subjects taking DTG and DRV (n=12),[305] in contrast to a 38% decrease seen in DTG C_{24} when co-prescribed with twice daily DRV/RTV in healthy volunteers.[306]

No intensive PK data have been published to date on DTG/DRV/COBI co-administration. We, therefore, aimed to describe the steady-state PK of DTG 50 mg (Tivicay®) OD and of fixed dose DRV/COBI 800/150 mg (Rezolsta®) OD, over 24 hours when co-administered in healthy volunteers.

4.2. METHODS

4.2.1. Participants

Eligible participants were male and non-pregnant and non-lactating female healthy volunteers aged between 18 and 65 years with a BMI between 18 and 35 kg/m^2. Participants were excluded if they had any significant acute or chronic medical illness; abnormal physical examination, ECG or clinical laboratory determinations; positive screens for HIV, hepatitis B or C; current or recent (within three months) gastrointestinal disease; clinically relevant alcohol or drug use which the investigator felt would adversely affect compliance with trial procedures; exposure to any investigational drug or placebo within three months of the first dose of the study drug; use of any other drugs, including over the counter medications and herbal preparations, within two weeks of the first dose of the study drug; and previous allergy to any of the constituents of the pharmaceuticals administered during the trial.

4.2.2. Study design

The study design is illustrated in **figure 4.1**. This was a randomised phase 1, open label, 57-day, crossover PK study carried out at the Clinical Trial Unit of the St. Stephen's Centre, Chelsea, and Westminster Hospital, London, United Kingdom. At screening, participants had a clinical assessment and routine laboratory investigations were performed. After successful screening, eligible participants were randomised to

one of two groups. Group one received DTG 50 mg OD for 14 days followed by a 7 day wash out (days 15 – 21). From day 22 to 35, the co-administration period, they received DTG 50 mg OD plus DRV/COBI 800/150 mg OD for 14 days, which was followed by a 7 day wash out (day 36 – 42) and finally a 14-day period of DRV/COBI 800/150 mg OD ensued. Group 2 followed the same structured sequence but started with DRV/COBI 800/150 mg OD and concluded with DTG 50 mg OD. Subjects were asked to take both DTG and DRV/COBI in the morning within 15 minutes of a standard breakfast. The safety and tolerability of study medications were evaluated throughout the trial (on days 7, 28, 49, PK days and at follow-up) using the NIAID Division of AIDS table for grading the severity of adult and paediatric adverse events (published in 2004). Each group underwent intensive PK sampling on study days 14, 35 and 56 to measure plasma concentrations of DTG and/or DRV/COBI at 0 (pre-dose), 2, 4, 8, 12- and 24-hours post-dose. On the PK days, study staff witnessed study medication intake with a standardized breakfast (626 kcal) and 240 mL of water.

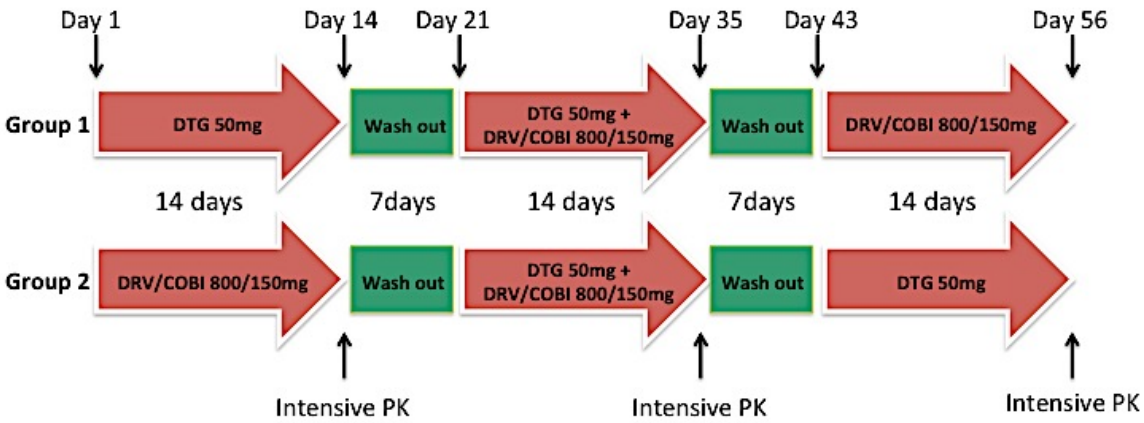


Figure 4.1: study design. PK = pharmacokinetics, DTG = dolutegravir, DRV = darunavir, COBI = cobicistat

4.2.3. PK sample collection

Blood samples were collected into lithium heparin-containing blood tubes (12 mL) at

each time-point, immediately inverted several times and then kept on ice or refrigerated until centrifugation. Within 30 minutes of blood collection, each blood sample was centrifuged for 10 min at 2000 g at 4°C. Plasma was then aliquoted equally into three 2.0 mL tubes (Sarstedt, Germany) and stored at -20°C. Samples were shipped on dry ice to the Liverpool Bioanalytical Facility for analysis. The laboratory is Good Clinical Laboratory Practice-accredited and participates in an external quality assurance scheme (KKGT, the Netherlands).[270, 271]

4.2.4. Quantification of plasma DTG, DRV and COBI

Concentrations of dolutegravir, darunavir and cobicistat in plasma were measured using validated high-pressure liquid chromatography–tandem mass spectrometry methods as previously described (HPLC MS/MS).[272, 273] The lower limits of quantification (LLOQ) for plasma DTG was 10 ng/mL, 15 ng/mL for DRV and 10 ng/mL for COBI. For concentrations below the assay limit of quantification, a value of one-half of the quantification limit was used. Accuracy (percentage bias) was between 92.5% and 96.2% (DTG), 104.1% and 104.9% (DRV) and 95.0% and 105.1% (COBI), and precision was between 2.6% and 4.1% (DTG), 4.4% and 9.4% (DRV) and 5.7% and 7.3% (COBI).

4.2.5. Data analysis

The calculated PK parameters for plasma DTG, DRV and COBI were C_{24}, C_{max} and $AUC_{0–24}$. All PK parameters were calculated using actual blood sampling time and non-compartmental modeling techniques (WinNonlin Phoenix, version 6.1; Pharsight, Mountain View, CA). Descriptive statistics, including GM 95% CI were calculated for DTG, DRV and COBI plasma PK parameters. Each drug PK parameter during the co-administration period was compared to the unaccompanied drug PK parameter by

calculating GM ratios (GMR) and 90% CI (co-administered/alone). Inter-individual variability in drug PK parameters was expressed as CV%.

Since both COBI and DTG are associated with a small rise in creatinine through renal MATE1 and OCT2 transporter inhibition respectively, the statistical significance of the changes in creatinine from baseline was calculated using the two-sided Wilcoxon signed-rank test for paired samples.[307, 308]

Additionally, as DTG is a substrate of P-gp and BCRP transporters, both of which are present within the endothelium of the blood brain barrier and are inhibited by COBI,[198] a review of neuropsychiatric adverse events (NP AE) was carried out.

4.2.6. Statistical power

This is an exploratory study and, as such, no formal sample size calculation was performed. Twenty (20) participants completing the study was deemed appropriate to allow for relevant conclusions, as is standard for PK studies.[309]

4.2.7. Ethics

The study protocol was approved by the Surrey Borders Research Ethics Committee and by MHRA UK. The study was conducted according to Good Clinical Practice and the Declaration of Helsinki (NCT03094507).

4.3. RESULTS

4.3.1. Study Population

Twenty-five healthy volunteers were screened, 21 attended baseline and 20 completed all PK phases (eleven in group 1 and 9 in group 2; one subject withdrew for personal

reasons). Median age was 33.5 years (range 24-63), 13 participants were female and median BMI was 27 (range 20-31) kg/m^2. Thirteen subjects described themselves as Caucasian, six as Black African/Caribbean and one as White and African.

4.3.2. DTG, DRV and COBI plasma pharmacokinetics

4.3.2.1. Dolutegravir plasma pharmacokinetics

Figure 4.2 illustrates the DTG GM plasma concentration *vs* time curves with and without DRV/COBI, in relation to DTG's PA-IC_{90} for wild type virus (64 ng/mL).[76] DTG geometric mean ratios (GMR, DTG+DRV/COBI *vs* DTG alone) and 90% confidence intervals (CI) for C_{max}, AUC_{0-24} and C_{24} were 1.01 (0.92-1.11), 0.95 (0.87-1.04) and 0.9 (0.8-1.0). No differences were seen between groups 1 and 2. The inter-individual variability in DTG values was between 23 and 40% when administered alone and between 28 and 48% during co-administration with DRV/COBI. C_{24} remained 7 to 32 fold above the PA-IC_{90}.[76]

4.3.2.2. Darunavir plasma pharmacokinetics

Figure 4.3 shows the DRV GM plasma concentration *vs* time curves with and without DTG in relation to DRV's suggested PA-EC_{50} for resistant virus (based on in-vitro studies; 550 ng/mL).[274] DRV GMR (DRV/COBI+DTG *vs* DRV/COBI alone) and 90% CI for C_{max}, AUC_{0-24} and C_{24} were 0.90 (0.83-0.98), 0.93 (0.86-1.00) and 0.93 (0.78-1.11) and for COBI C_{max}, AUC_{0-24} and C_{24} were 0.96 (0.89-1.04), 0.98 (0.88-1.08) and 0.98 (0.79-1.22). The inter-individual variability in DRV values was between 31 and 52% when administered alone and between 20 and 53% during DTG co-administration.

C_{24} remained 1 to 4.5 fold above the suggested PA-EC_{50} (550 ng/mL)[274] for DRV in

all subjects (except for one participant with a DRV C_{12} of 1428 ng/mL but C_{24} 185 ng/mL).

Table 4.1 summarises the PK parameters for DTG and DRV/COBI when administered alone or together in the co-administration phase, in both groups combined. No difference was seen between groups for either drugs.

4.3.3. Safety and tolerability

The studied drugs were well tolerated, with no grade 3 or 4 side effects or laboratory abnormalities. Median (IQR) creatinine was 67 μmol/L (63-71) at baseline, 70 μmol/L (65-74) during DRV/COBI alone, 76 μmol/L (69-81) during DTG alone and 74.5 μmol/L (70-79.5) during co-administration (equivalent to 0.75,0.79, 0.86 and 0.84 mg/dL respectively). The difference between baseline and during co-administration was significant (T=2.5, p <0.01), which was driven by DTG. However, there was no evidence that adding DRV/COBI to DTG changed median creatinine significantly (>0.05), whilst adding DTG to DRV/COBI did (T=29.5, p <0.01).

Grade 1-2 drug related NP-AEs were seen in 6 participants (30%) during DTG monotherapy and in 3 participants (15%) during DRV/COBI monotherapy. The co-administration of the two drugs did not change the prevalence of drug related NP-AEs, which was 15% during the co-administration period and remained low grade (1-2). The commonest drug related NP-AEs were a grade 1 headache and sleep disturbances.

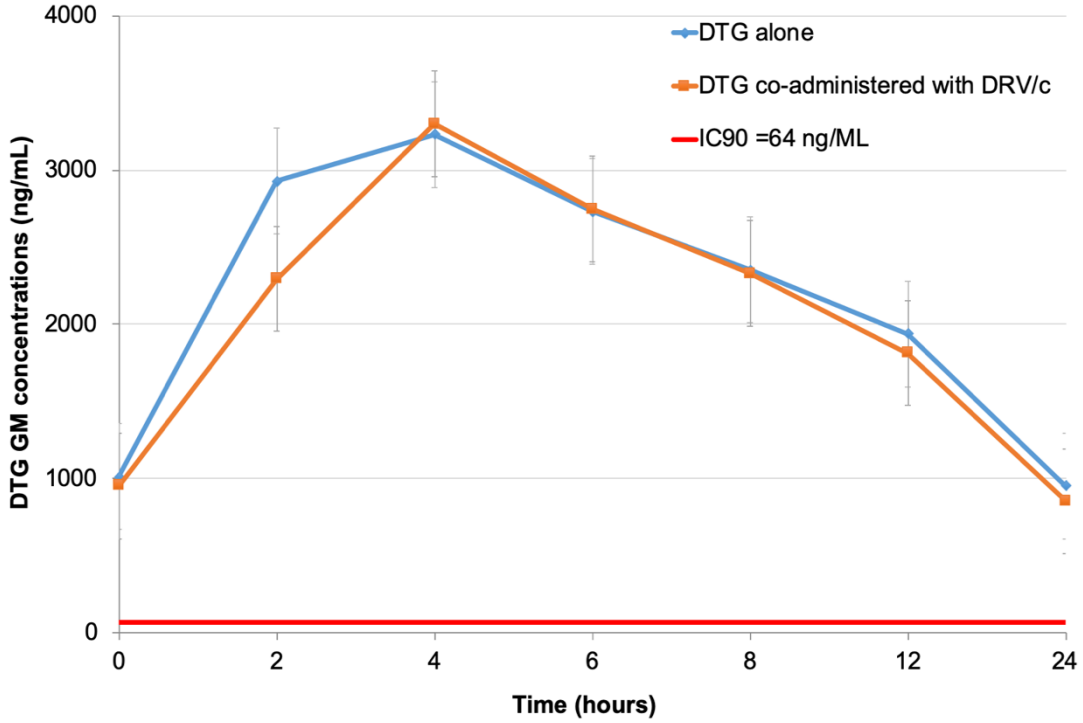


Figure 4.2: GM dolutegravir concentration *vs* time curves and standard error bars over 24 hours with and without darunavir/cobicistat (DRC/c)

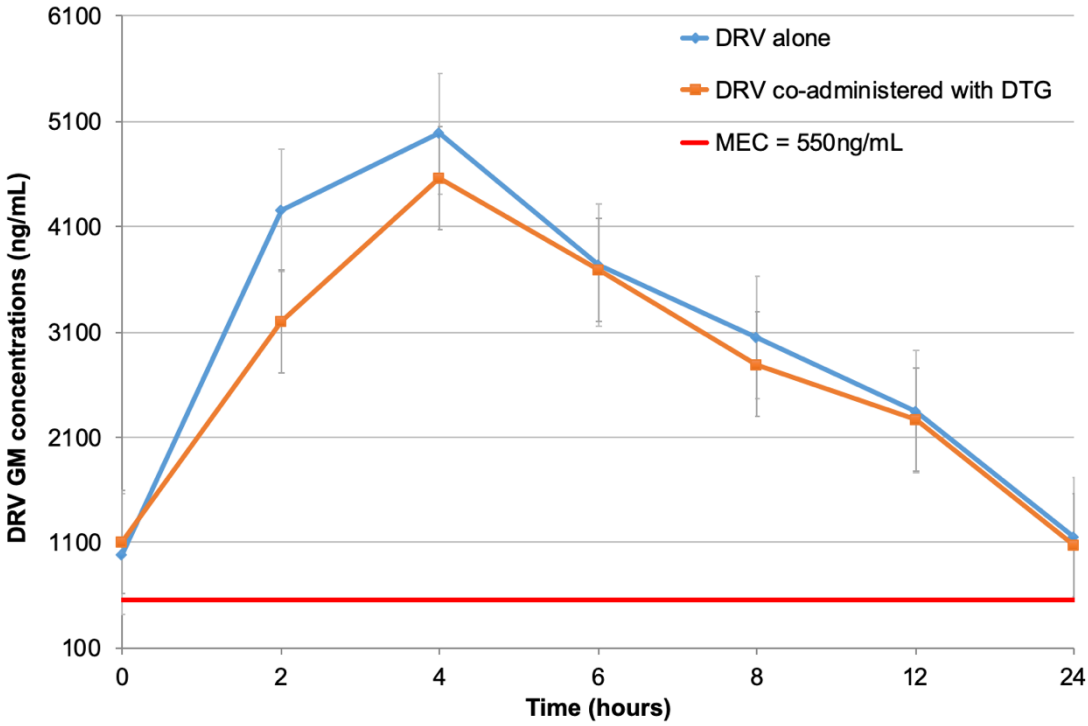


Figure 4.3: GM darunavir concentration *vs* time curves and standard error bars over 24 hours with and without dolutegravir

	GM C_{max} (90% CI) (ng/mL)					GM AUC_{0-24} (90% CI) (ng*h/mL)					GM C_{24} (90% CI) (ng/mL)				
	Alone	**CV%**	**Combined**	**CV%**	**GMR**	**Alone**	**CV%**	**Combined**	**CV%**	**GMR**	**Alone**	**CV%**	**Combined**	**CV%**	**GMR**
DTG	3398 (3087-3708)	23	3429 (3104-3755)	46	1.01 (0.92-1.11)	47669 (42377-52960)	28	45188 (40203-50174)	28	0.95 (0.87-1.04)	952 (795-1109)	40	852 (690-10145)	46	0.9 (0.80-1.00)
DRV	5364 (4726-6003)	31	4821 (4455-5187)	20	0.90 (0.83-0.98)	63222 (55152-71291)	33	58864 (52978-64750)	26	0.93 (0.86-1.00)	1146 (891-1400)	52	1070 (817-1322)	53	0.93 (0.78-1.11)
COBI	967 (868-1066)	27	929 (845-1014)	24	0.96 (0.89-1.04)	7829 (6865-8793)	32	7650 (6619-8682)	35	0.98 (0.88-1.08)	19 (10.4-28)	90	19 (6.7-31)	111	0.98 (0.79-1.22)

Table 4.1: Dolutegravir (DTG), darunavir (DRV) and cobicistat (COBI) steady state pharmacokinetic (PK) parameters, expressed as geometric mean (GM), 90% confidence intervals (CI), coefficient of variation (CV) and GM Ratios (GMR, alone/co-administered). C_{max}: maximum concentrations, AUC_{0-24}: area-under-the-curve, C_{24}: concentration measured 24 hours post-dose.

4.4. DISCUSSION

The steady state PK of standard dose DTG co-administered with DRV/COBI over 24 hours was characterised in healthy volunteers. The changes in DTG PK parameters during co-administration compared to DTG administered alone were minimal. DTG C_{24} decreased by 10%, whilst AUC_{0-24} decreased by 5% and C_{max} remained unchanged. DRV concentrations also decreased by less than 10% in all parameters. DTG and DRV concentrations remained manifold above the PA-IC_{90}/PA-EC_{90} in all participants at all time points, suggesting that the combination of DTG and DRV/COBI can be prescribed safely in the treatment of HIV-1, including in patients harbouring resistance who benefit from optimal ARV exposures and in which BD dosing is recommended. In contrast, DTG C_{24} had decreased by 38% when co-administered with DRV/RTV (twice daily) in early DTG drug interaction studies, which was not deemed clinically significant (C_{max} decreased by 11% and AUC_{0-24} 22%; participants received multi-dose DTG 30 mg, administered with food).[306]

Our findings are in agreement with Gervasoni *et al.*, who showed a doubling of DTG C_{24} in HIV patients who switched from DRV/RTV to DRV/COBI.[305] DTG is primarily metabolised by UGT1A1 and is only a minor substrate for CYP3A4.[172] Whilst both COBI and RTV are potent CYP3A4 inhibitors, unlike RTV, COBI does not induce glucoronidation (or any CYP enzymes),[196] which, as previously discussed, is likely to explain the difference in effect seen between the two pharmacological boosters. A lack of inhibition of UGTA1A1, 1A3, 1A6, 1A9, 2B4 and 2B7, by both RTV and COBI, was recently demonstrated in-vitro, meaning that, in agreement with our results, COBI has a lack of effect overall on UGT.[310] Interestingly, Gervasoni *et al.* commented on

the possibility that their observed surge in DTG concentrations when RTV was switched to COBI may be driven, at least in part, by a higher degree of inhibition of COBI on intestinal efflux transporters (P-gp and BCRP), leading to increased DTG absorption.[196, 305] There was no rise in DTG C_{max}, in our study, when DRV/COBI was added to DTG, which would suggest, conversely, that the inhibitory effect of COBI on these transporters in this setting is, at the most, limited.[196, 198] DTG does not induce or inhibit CYP enzymes,[76] therefore effects on DRV and COBI (which are mainly metabolised by CYP3A4) were not expected during co-administration with DTG.

Interestingly, the PK parameters of DTG in this study were, overall, lower than seen in Min *et al.*'s early DTG PK study (healthy volunteers, n=8, food intake not specified) when administered alone and lower than in the Gervasoni *et al*. study when co-administered with COBI, highlighting the importance of describing intra-individual effect in drug interaction studies.[60, 305]

Serum creatinine concentrations significantly increased from baseline during DTG administration, but no significant increment was recorded when DRV/COBI was added to DTG, which is consistent with previous observations that administration may not result in additive renal effect, at least in the short-term.[305, 308] The commonest NP-AEs seen were mild headache and sleep disturbances, particularly with DTG (30%). Co-administration of DTG and DRV/COBI did not increase the prevalence of NP-AEs, however, this study was not powered for a toxicodynamic analysis.

There are limitations to this study. Subjects were healthy volunteers and pharmacokinetic conclusions cannot be fully drawn in HIV infected participants. In

licencing trials, DTG concentrations appeared generally lower in HIV infected participants than in healthy volunteers.[57] Indeed, discrepancies in ARV drug PK between healthy volunteers and PLWH have been previously described (particularly for the protease inhibitors), which was discussed in **chapter 3**.[288] Additionally, pharmacodynamics deductions cannot be drawn from healthy volunteers; however, previous cohort studies have reported good efficacy of DTG/DRV/COBI in small groups of treatment experienced HIV infected individuals.[119, 120] Finally, DTG intensive PK over 24hrs remain to be determined during co-administration with DRV when the latter is boosted with RTV (to date only 12hr data is available); this is important in view of RTV's known induction of UGT1A1 and is particularly relevant to patients with a background of InSTI resistance.

The strengths of this study lie in its prospective, controlled and crossover design, which allowed an analysis of *intra-individual* effect. Additionally, the study population was appropriately diverse in gender, ethnicity and age.

In conclusion, this study investigated the intra-individual variance in DTG and DRV/COBI PK parameters when administered together compared to alone. The results suggest that no dose adjustment is required in either agents and that this combination can be prescribed safely, at standard recommended doses, in the treatment of HIV-1, including in patients harbouring resistance, when BD dosing remains standard practice.

CHAPTER 5

Pharmacokinetics Of Ethinylestradiol/Levonorgestrel Co-administered With Atazanavir/Cobicistat

CITATION

Elliot ER, Bisdomini E, Penchala SD, Khoo S, Nwokolo N, Boffito M. Pharmacokinetics (PK) of ethinylestradiol/levonorgestrel co-administered with atazanavir/cobicistat. HIV Res Clin Pract. 2019 Jul 23:1-10. doi: 10.1080/25787489.2019.1638077

CONTENT

5.1. INTRODUCTION

Women account for just over half of the world's 36.7 million people living with HIV/AIDS and the majority are of childbearing age.[311] Early and sustained HIV viral load suppression with ARV therapy now enables longer, healthier lives and improved fertility in women living with HIV (WLWH).[312-314] A significant number do, however, report requiring the flexibility to plan or prevent pregnancy,[313, 314] meaning that access to safe and reliable contraception in the context of ARVs is critical.[315, 316] The combined oral contraceptive pill (COCP) is a preferred method of contraception for many women worldwide.[317, 318] Nevertheless, WLWH have often been unable to use the COCP due to drug-drug interactions with some ARVs leading to altered drug levels and adverse events.[319] For instance, a reduction in the progestogen component of the COCP potentially runs the risk of contraceptive failure and unwanted pregnancy[319] whilst overexposure can lead to a number of side effects such as increased appetite, fluid retention, acne and headaches.[320] Meanwhile, changes in oestrogen pharmacokinetics impact tolerability (e.g. breakthrough bleeding with underexposure, which can impact adherence) and toxicity (e.g. thrombotic events with increased concentrations).[321-323] There is now good evidence that an undetectable viral load equates to HIV being untransmittable[4] and, hence, many HIV serodifferent couples choose to rely on hormonal contraception alone without condoms.[313, 324] It is therefore essential to define the pharmacokinetics and pharmacodynamics of hormonal contraception co-administered with individual ARVs in order to inform guidelines and ensure that the efficacy and safety of both are maintained.[321]

In addition, exogenous oestrogens are also used by trans-gender women (TGW) as

feminising therapy.[325] TGW carry a disproportionate burden of HIV infection (estimated global prevalence 19.6% and incidence 3.4 to 7.8 per 100 person-years worldwide)[326, 327] and are less likely to engage into care or being compliant with ART.[328, 329] Qualitative studies have attributed this, in parts, to a limited understanding of the interactions between some ARVs and gender affirming hormones and to fears amongst TGW that ARVs will impede on hormonal efficacy, further underscoring the importance of having evidence-based data on DDIs.[325, 330] Ethinylestrodiol (EE), specifically, is not recommended for feminisation therapy in guidelines;[331] however, it is the most studied oestrogen and the reference agent for drug interaction guidance.[325] It is also often used by TGW outside of healthcare settings for medical transition.[332]

Despite very recently being removed from some major guidelines,[312, 333, 334] boosted atazanavir (ATV/b) remains the preferred second-line ARV agent in the World Health Organisation guidelines and large numbers of patients, stable on treatment, continue therapy with ATV/b worldwide.[246] With its long-standing experience history, high genetic barrier and once daily dosing, it remains an instrumental option in the management of HIV.[335] For over a decade, ATV was mainly co-administered with the only pharmacological booster available, RTV. Hormonal contraceptives are extensively metabolised by CYP enzymes (CYP3A and CYP2C9/19) and drug interactions between the COCP and ATV (with or without RTV) have been demonstrated.[336] The progestogens studied to date, norgestimate (NGM) and norethisterone (NET), are both increased in exposure with unboosted and with ritonavir-boosted ATV, through the inhibition of CYP3A4-mediated progestogen metabolism by both agents, thereby potentially leading to the side effects described

above.[337, 338] Similarly EE concentrations also increase with unboosted ATV (48% rise in area under the curve, AUC)[339] but, in contrast, decrease with ATV/RTV (16% decrease in EE C_{max}, 19% in AUC and 37% in C_{min})[338] which is thought to result from RTV's concomitant induction of CYP2C9 and of the glucuronidation responsible for EE clearance (via UGT1A1).[336] Therefore, according to guidelines and the ATV summary of product characteristics (SPC), if a combined oral contraceptive is administered with ATV/RTV, it must contain at least 35µg of EE, strict compliance is necessary and a second method of contraception is recommended, considering the unknown PD effect of the drug interactions.[255, 338, 339]

Unlike RTV and as previously discussed, COBI is available co-formulated with ATV (Evotaz®) and is neither a UGT1A1 nor a CYP2C9 inducer.[198-200] The product insert for Evotaz® and the University of Liverpool's HIV drug interactions website[58] state that no dosing recommendations can be made for COCP co-administration with ATV/COBI due to a lack of published data. It is currently suggested that additional or alternative (non-hormonal) forms of contraception should be considered. ATV/COBI co-administration with the COCP has been investigated in an unpublished, phase I drug interaction study (Majeed *et al.*, IWCPAT, Chicago 2017), which only assessed a single dose of drospirenone (DRSP)/EE 3mg/20µg administered prior to and at the end of a 14-day course of standard dose ATV/COBI in healthy volunteers (n=14). The investigators found a 130% increase in DRSP $AUC_{0-\infty}$ and a 12% increase in C_{max}. There was a non-clinically significant decrease in EE (22% reduction in $AUC_{0-\infty}$ and 18% in C_{max}). C_{24} was not reported.[340] Neither COCP C_{24} nor '*steady state'* (real life use) PK data are available; the latter are important since progestogen serum levels are 2 to 3 fold higher in the steady state compared to a single administration (after

approximately 8-10 days of treatment) and EE steady state concentrations increase by a 30-40% rise in plasma level (5-6 days post-initiation).[323, 341]

EE/levonorgestrel (LNG; Microgynon®) is the leading COCP prescribed in the UK.[342] The aim of this study was therefore to investigate the steady state PK of EE/LGN 30/150µg and ATV/COBI 300/150mg (Evotaz®) when co-administered in HIV negative female healthy volunteers and to assess the safety and tolerability of co-administration.

5.2. METHODS

5.2.1. Participants

Written informed consent was obtained from non-pregnant and non-lactating female healthy volunteers aged between 18 and 35 years with a body mass index (BMI) between 18 and 35 kg/m^2. Participants were excluded if they had any significant acute or chronic medical illness; abnormal physical examination, ECG or clinical laboratory determinations; positive screens for HIV, hepatitis B or C; current or recent (within three months) gastrointestinal disease; clinically relevant alcohol or drug use that the investigator felt would adversely affect compliance with trial procedures; exposure to any investigational drug or placebo within three months of the first dose of the study drug; use of any other drugs, including over the counter medications and herbal preparations, within two weeks of the first dose of the study drug; and previous allergy to any of the constituents of the pharmaceuticals administered during the trial. Women of childbearing potential required a negative pregnancy test at screening and baseline and additional contraception if required.

5.2.2. Study design

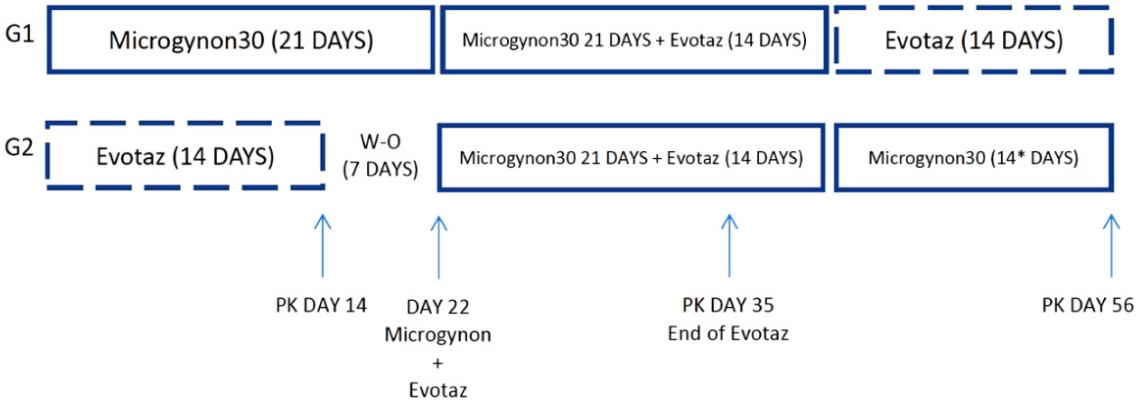


Figure 5.1: Study design. PK: Pharmacokinetics, W-O: Washout period. *14 or 21 days, patient choice

The study design is shown in **figure 5.1.** This was an open-label, crossover, 57-day (excluding screening and follow-up) phase 1 PK trial carried out at the Clinical Trial Unit of the St. Stephen's Centre, Chelsea and Westminster Hospital, London, UK.

At screening, clinical assessment and routine laboratory investigations were performed in all participants. The safety and tolerability of study medications were evaluated throughout the trial (on days 7, 28, 49, on PK days and at follow-up) using the previously referred to NIAID Division of AIDS table for grading the severity of adult and pediatric adverse events (2004). After successful screening, volunteers were randomized to either i) group 1, which received EE/LNG 30/150 µg alone on days 1-21, EE/LNG (21 days) + ATV/COBI 300/150 mg (14 days) in the co-administration period (days 22-42) and ATV/COBI alone on days 43-56 (14 days) or ii) group 2, which followed the same structured sequence but started with ATV/COBI alone and finished with EE/LNG alone (14 or 21 days, patient choice). Each group underwent intensive PK sampling on study days 14, 35 and 56 to measure plasma concentrations of EE/LNG and/or ATV/COBI at 0 (pre-dose), 2, 4, 8, 12- and 24-hours post-dose. On the PK days, study staff witnessed study medication intake with a standardized breakfast (626 kcal) and 240 mL of water.

Adherence:

Adherence was assessed through direct questioning of dosing schedules, missed and late doses at each safety and PK visit. A pill count was carried out at each PK visit.

5.2.3. Analytical and PK methods

Blood samples were collected into lithium heparin-containing blood tubes (12 mL) at each time-point, immediately inverted several times and then kept on ice or refrigerated until centrifugation. Within 30 minutes of blood collection, each blood sample was centrifuged for 10 minutes at 2000 *g* at 4°C. Plasma was then aliquoted equally into three 2.0 mL tubes (Sarstedt, Germany) and stored at -20°C. Samples were shipped on dry ice to the Liverpool Bioanalytical Facility for analysis. The laboratory is Good Clinical Laboratory Practice-accredited and participates in an external quality assurance scheme (KKGT, the Netherlands).[270, 271]

5.2.4. Quantification of LNG, EE, ATV and COBI

Concentrations of LNG, EE, ATV and COBI in plasma were measured using validated high-pressure liquid chromatography–tandem mass spectrometry methods (LC-MS/MS).[272, 273, 343] The LLOQ for the plasma analyses were 0.240 ng/mL for LNG, 5 pg/mL for EE, 10 ng/mL for ATV, and 5 ng/mL for COBI. For concentrations below the assay limit of quantification, a value of one-half of the quantification limit was used. Accuracy (percentage bias) was between -0.42% and 1.5% (LNG), 0.61% and 3.28% (EE), 4.70% and 6.36% (ATV), and 6.45% and 8.07% (COBI) and precision was less than 3.1% (LNG), 8.0% (EE), 6.3% (ATV), and 8.0% (COBI).

5.2.5. Data analysis

The calculated PK parameters for plasma LNG, EE, ATV and COBI were plasma C_{24}, C_{max} and AUC_{0-24}. All PK parameters were calculated using actual blood sampling time and non-compartmental modeling techniques (WinNonlin Phoenix, version 6.1; Pharsight, Mountain View, CA). Descriptive statistics, including GM 90% CI were calculated for LNG, EE, ATV and COBI plasma PK parameters. Each drug PK parameter during the co-administration period was compared to the unaccompanied drug PK parameter by calculating GMR and 90% CI (co-administered/alone). Inter-individual variability in drug PK parameters was expressed as CV%.

5.2.6. Statistical power

This was an exploratory study and, as such, no formal sample size calculation was performed. Sixteen (16) participants completing the study was deemed appropriate to allow for relevant conclusions and is standard for PK studies.

5.2.7. Ethics

The study protocol was approved by the Westminster Research Ethics Committee, London, United Kingdom, as well as by MHRA UK. The study was conducted according to Good Clinical Practice and the Declaration of Helsinki (NCT02697851).

5.3. RESULTS

5.3.1. Study population

Fourteen healthy female volunteers were screened, 13 were enrolled and 11 attended the baseline visit (2 withdrew prior to baseline for personal reasons). 9 completed the intensive PK day 14 (five in group 1, four in group 2), 8 completed day 35 (four in each group) and 6 completed day 56 (four in group 1, two in group 2). Overall, seven

participants withdrew consent; five because of adverse events and two before starting the study medications. Of the six participants who completed all PK phases (up to day 56), the median (range) age and BMI were 31 (19-35) years, and 24 (19-29) kg/m^2, respectively. Of the eleven participants who attended the baseline visit, four participants described themselves as Caucasian, six as black African, and one as Hispanic. Demographics and reasons for withdrawals are summarised in **table 5.1.**

ID	Age (yrs)	Ethnicity	BMI	Group	Study stage reached	Reason for withdrawal
101	31	Hispanic	21.1	G1	Completed	N/A
102	27	White	23.7	G2	Between D7 and D14	Rash
103	30	African	29.3	G1	Completed	N/A
104	33	White	22.1	G2	Completed	N/A
105	30	African	26.8	G1	Between D28 and D35	Deranged LFTs
106	28	Turkish	23.1	G1	Before baseline	1st dose not given
107	35	White	26.9	G1	Completed	N/A
108	23	WBC	27.4	G2	Between D35 and D43	Moderate nausea, D&V, orthostatic hypotension
109	18	White	20.7	G2	Between Baseline and D7	Side effects, not specified
110	27	White	25.3	G2	Completed	N/A
111	19	White	19.0	G1	Completed	N/A
112	25	WBC	22.4	G2	Between D35 and D43	No cause given
113	29	White	27.6	G1	Before baseline	1st dose not given

Table 5.1: Participant demographics, withdrawals and withdrawal reasons. ID = study identification, BMI = body mass index, G = group, D = day, n/a = not available, WBC = White and Black Caribbean

5.3.2. Pharmacokinetic results

PK data for all four drugs in the two separate groups are detailed in **table 5.2**. For each drug, only paired data from participants who completed the first period (either EE/LNG or ATV/COBI alone) and the co-administration period were used, so as to be able to assess and summarise intra-individual PK changes between the two periods. Groups 1 and 2 (G1 and G2) are grouped together in the results described below.

5.3.2.1. Levonogestrel plasma pharmacokinetics

Six participants provided data for LNG intake with and without ATV/COBI (G1 n=4, G2 n=2). Geometric mean ratios (with ATV/COBI *vs* without) and 90% CI for LNG C_{max}, AUC_{0-24} and C_{24} were 0.83 (0.68-1.02), 0.92 (0.71-1.18) and 1.01 (0.73-1.38) respectively.

5.3.2.2. Ethinylestradiol plasma pharmacokinetics

Six participants provided data for EE intake with and without ATV/COBI (G1 n=4, G2 n=2). GMR (90% CI) for EE C_{max}, AUC_{0-24} and C_{24} were 1.05 (0.92-1.19), 1.01 (0.88-1.22) and 0.75 (0.60-0.93) respectively.

5.3.2.3. Atazanavir plasma pharmacokinetics

Eight participants provided data for ATV intake with and without EE/LNG (G1 n=4, G2 n=4). GMR (90% CI) of ATV C_{max}, AUC_{0-24} and C_{24} were 0.75 (0.60-0.95), 0.78 (0.64-0.96) and 0.89 (0.72-1.11) respectively.

5.3.2.4. Cobicistat plasma pharmacokinetics

Eight participants provided data for COBI intake with and without EE/LNG (G1 n=4, G2 n=4). GMR (90% CI) of COBI C_{max}, AUC_{0-24} and C_{24} were 0.88 (0.8-0.97), 0.85 (0.77-0.95) and 0.89 (0.66-1.21) respectively.

LNG and EE drug concentrations *vs* time curves with and without ATV/COBI are illustrated in **figures 5.2** and **5.3** respectively**. Table 5.3** summarises the results in this study together with currently reported findings of COCP interaction studies involving ATV/RTV and/or COBI.

	GM C_{max} (90% CI) (ng/mL)					GM AUC_{0-24} (90% CI) (ng*h/mL)					GM C_{24} (90% CI) (ng/mL)				
	Alone	**CV%**	**Combined**	**CV%**	**GMR**	**Alone**	**CV%**	**Combined**	**CV%**	**GMR**	**Alone**	**CV%**	**Combined**	**CV%**	**GMR**
LNG (n=6)															
Total	6.5 (3.5-9.6)	60	5.4 (3.7-7.2)	43	0.83 (0.68-1.02)	92 (41-143)	67	84 (57-111)	43	0.92 (0.71-1.18)	2.83 (1.2-4.5)	67	2.85 (1.9-3.8)	45	1.01 (0.73-1.38)
Group 1 (n=4)	8.8 (5.9-12)	46	7.2 (6.1-8.3)	23	0.82 (0.61-1.11)	130 (81-180)	50	111 (93-129)	23	0.85 (0.64-1.12)	4.2 (2.7-5.7)	47	3.8 (3.2-4.5)	25	0.90 (0.64-1.27
Group 2 (n=2)	3.6 (1.5-5.7)	47	3.09 (2.1-4.1)	26	0.86 (0.66-1.12)	45 (3.2-87)	69	48 (36-61)	23	1.07 (1.74-2.69)	1.3 (-0.05-2.6)	75	2.6 (1.0-4.2)	59	0.9 (0.64-1.27)
EE (n=6)															
Total	40 (28-53) $x10^{-3}$	43	42 (32-52) $x10^{-3}$	34	1.05 (0.92-1.19)	407 (259-555) $x10^{-3}$	49	410 (318-501) $x10^{-3}$	32	1.01 (0.83-1.22)	7.4 (3-12) $x10^{-3}$	72	5.6 (3.9-7.3) $x10^{-3}$	42	0.75 (0.6-0.93)
Group 1 (n=4)	43 (28-58) $x10^{-3}$	47	46 (32-60) $x10^{-3}$	35	1.07 (0.88-1.3)	446 (281-612) $x10^{-3}$	50	440 (335-544) $x10^{-3}$	33	0.98 (0.77-1.26)	8.2 (3-13) $x10^{-3}$	76	5.5 (3.7-7.3) $x10^{-3}$	45	0.67 (0.50-0.89)
Group 2 (n=2)	36 (29-42) $x10^{-3}$	26	36 (32-39) $x10^{-3}$	15	1 (0.88-1.13)	338 (202-475) $x10^{-3}$	55	356 (302-411) $x10^{-3}$	22	1.05 (0.7-1.59)	6.1 (3.2-9) $x10^{-3}$	63	5.7 (3.5-8) $x10^{-3}$	53	0.94 (0.82-1.08)
ATV (n=4)															
Total	4157 (3151-5163)	39	3133 (2472-3795)	34	0.75 (0.60-0.95)	48998 (37387-60608)	38	38293 (27206-49380)	45	0.78 (0.64-0.96)	1061 (614-1507)	62	949 (527-1371)	66	0.89 (0.72-1.11)
Group 1 (n=4)	4447 (2692-6201)	45	2861 (2158-3565)	29	0.64 (0.46-0.90)	49455 (35411-634500)	33	33680 (24048-43312)	32	0.68 (0.51-0.91)	930 (348-1510)	65	787 (543-1031)	35	0.85 (0.59-1.21)
Group 2 (n=4)	3886 (2692-5080)	36	3432 (2286-4577)	38	0.88 (0.65-1.21)	48544 (27777-69312)	48	43539 (23393-63684)	50	0.90 (0.69-1.17)	1211 (467-1954)	65	1145 (341-1949)	71	0.95 (0.71-1.27)
COBI (n=4)															
Total	1760 (1604-1917)	15	1554 (1325-1782)	25	0.88 (0.8-0.97)	16456 (13863-19049)	26	14054 (10703-17405)	39	0.85 (0.77-0.95)	70 (27-113)	84	62 (13-112)	101	0.89 (0.66-1.21)
Group 1 (n=4)	1810 (1669-1952)	9	1414 (1173-1655)	20	0.78 (0.7-0.87)	15834 (13044-18624)	21	11820 (9572-14069)	23	0.75 (0.65-0.86)	49 (25-73)	53	44 (26-61)	44	0.89 (0.47-1.67)
Group 2 (n=4)	1712 (1409-2014)	21	1707 (1332-2082)	26	1 (0.95-1.05)	17102 (12363-21842)	32	16710 (10942-22479)	40	0.98 (0.91-1.05)	99 (21-177)	78	89 (-4.7-182)	94	0.90 (0.74-1.09)

Table 5.2: Summary of pharmacokinetic data for all four drugs. EE = ethinylestradiol, LNG = levonorgestrel, ATV = atazanavir, COBI = cobicistat, CV = coefficient of variation, GM = geometric mean, GMR = geometric mean ratio, CI = confidence interval, Cmax = maximum concentration, AUC_{0-24} = area under the curve from 0 to 24 hours, C_{24} = concentration at 24 hours post-dose.

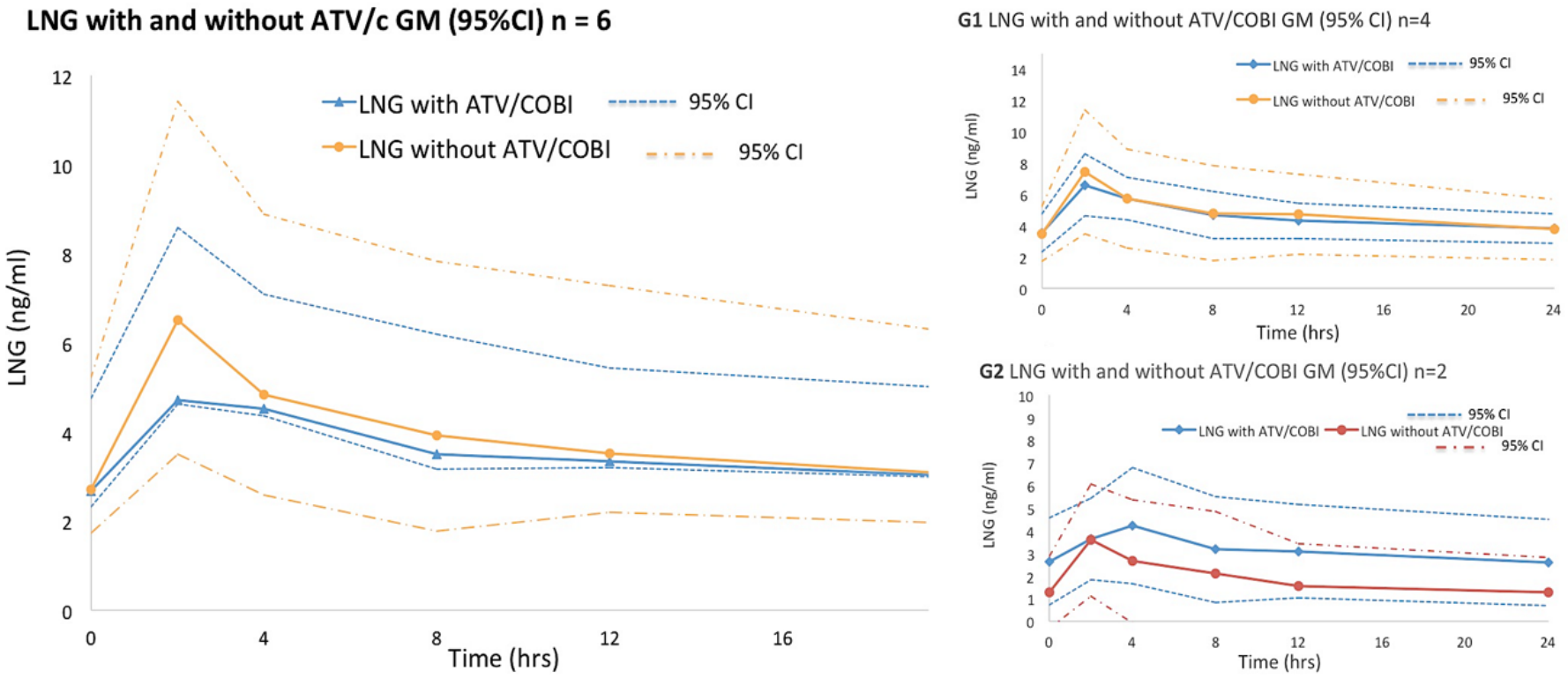


Figure 5.2: Levonogestrel (LNG) GM (95% CI) plasma concentration *vs* time curves, with and without ATV/COBI, GM (95% CI) n=6. LNG = levonorgestrel, ATV = atazanavir, COBI = cobicistat, GM = Geometric Mean, CI = Confidence Interval

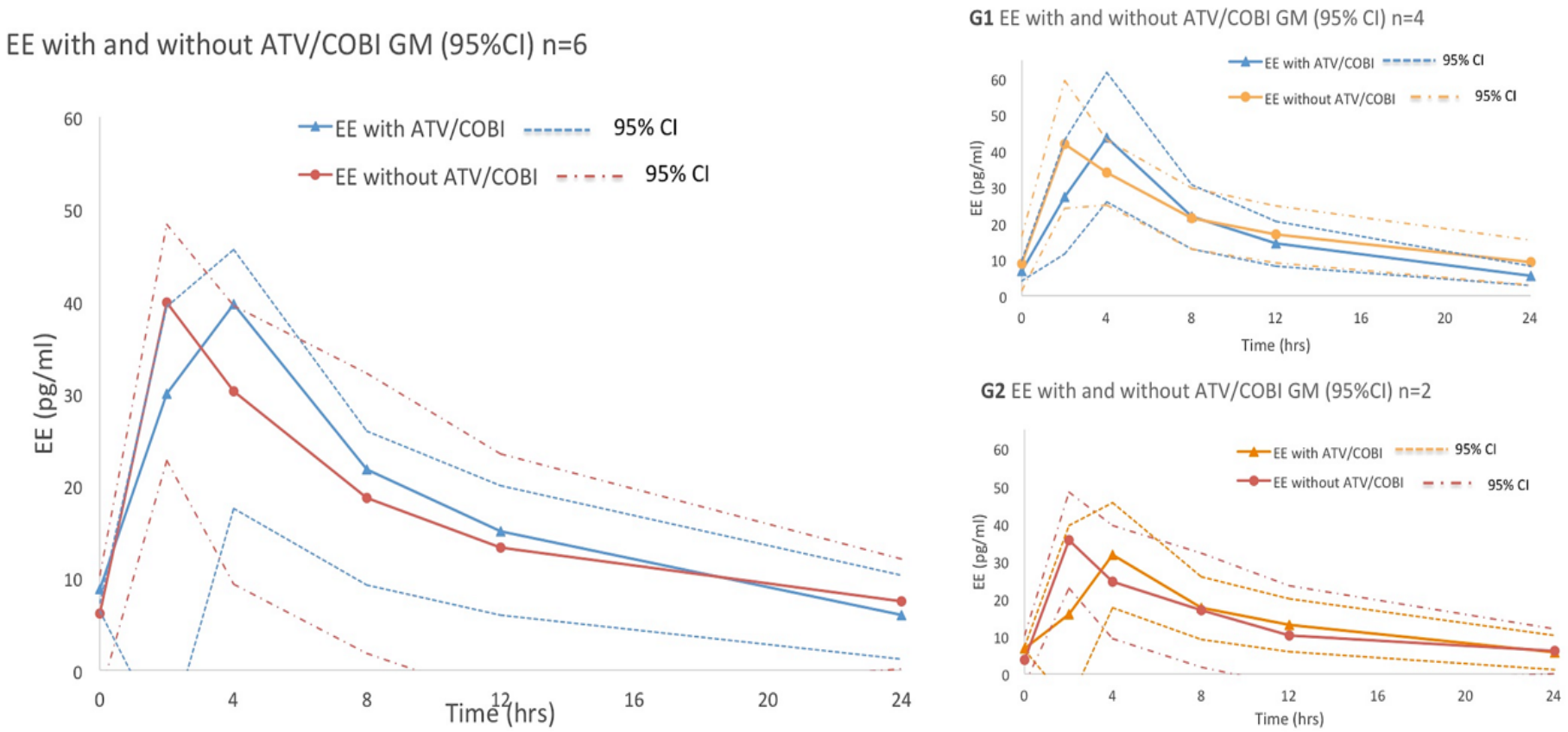


Figure 5.3: Ethinylestradiol EE GM (95% CI) plasma concentration *vs* time curves, with and without ATV/COBI, GM (95% CI) n=6. EE = ethinylestradiol, ATV = atazanavir, COBI = cobicistat, GM = Geometric Mean, CI = Confidence Interval

		ATV/COBI + EE/LNG (this study)	PI/RTV + NET[a] (n=16)	ATV/RTV + EE/NGM[b] (n=13)	TDF/FTC/ EVG/COBI + EE/LNG[c] (n=20)	ATV/COBI + EE/DRSP[d] (n=14)
Progestogen	**AUC**	↓ 8%	↑ 50%	↑ 85%	↑ 110%	↑ 130% ($AUC_{0-\infty}$)
	C_{max}	↓ 17%	↑ 33%	↑ 68%	↑ 60%	↑ 12%
	C_{min}	↑ 1%	↑ 26%	↑ 102%	N/A	N/A
EE	**AUC**	↑ 1%	N/A	↓ 19%	↑ 20%	↓ 22% ($AUC_{0-\infty}$)
	C_{max}	↑ 5%	N/A	↓ 16%	↑ 10%	↓ 18%
	C_{min}	↓ 25%	N/A	↓ 37%	N/A	N/A

Table 5.3: Summary of COCP drug interactions studies with atazanavir, ritonavir & cobicistat. Legend: AUC = Area Under the Curve; C_{max} = maximum concentration reached; C_{min} = trough concentration; ATV= atazanavir; COBI = cobicistat; EE = ethinylestradiol; LNG = levonogestrel; PI = protease inhibitor; RTV = ritonavir; NET: norethindrone; NGM = norgestimate; TDF = tenofovir; FTC = emtracitabine; EVG = elvitegravir; DRSP = drospirenone; N/A: not applicable.

a: Atrio *et al*. J Acquir Immune Defic Syndr. 2014[337] **b**: Zhang *et al*. Antivir Ther. 2011[338] **c:** Squires *et al*. Asia Pacific AIDS Conference. 2016[344] **d**: Majeed *et al*. IWCPAT. 2017[340]

5.3.3. Safety and tolerability

Five participants withdrew consent from the study secondary to side effects; of those, data on the reason are available for three and are listed in **table 5.1**. Full adherence was confirmed through regular direct questioning and pill counts in the women who completed the study and no grade 3 or 4 adverse events or laboratory abnormalities were observed in this group.

5.4. DISCUSSION

This was the first study to investigate the steady state PK of Microgynon® co-administered with ATV/COBI (Evotaz®). The hormonal components of the COCP undergo extensive first-pass metabolism by phase I and II microsomal enzymes in the small intestinal mucosa and the liver before reaching the systemic circulation, meaning

that they are highly susceptible to DDI.[345, 346] LNG is first hydroxylated in the liver, mainly by CYP3A4, and its metabolites are then excreted as glucuronide conjugates.[347] The results of this study showed only a small *reduction* in steady state LNG C_{max} (17%) and no changes in C_{24} or $AUC_{0\text{-}24}$ when it is administered with ATV/COBI (GMR range 0.92-1.01). Majeed *et al.* had also reported little interaction with progestogens, with only a 12% *increase* seen in DRSP C_{max} when co-administered as a single dose with ATV/COBI. Both studies therefore suggest that ATV/COBI has a lesser impact on progestogen peak concentrations than ATV/RTV does when co-administered with NGM or NET (GMR 1.33-1.68)[337, 338, 340] and when NGM is co-administered with COBI as part of Stribild® (TDF/FTC/EVG/COBI; GMR 2.08; Polina *et al*. 12th Clin Pharm of HIV Therapy Workshop 2011). Whilst the number of participants completing the relevant phases in our study (n=6) was small and our data cannot be conclusive, a lack of clinically significant decrease in LNG minimum concentration and exposure is cautiously reassuring. This is because the progestogen-mediated suppression of the luteinising hormone (LH) surge is one of the main contraceptive mechanisms of the COCP and LNG is also the progestogen contained in the most commonly prescribed form of the emergency contraceptive pill.[347-349] Furthermore, the lack of meaningful rise in progestogen C_{24} and $AUC_{0\text{-}24}$ in the study presented here differs to the increases seen with ATV/RTV co-administration (GMR 1.85 and 2.02 respectively) or with COBI co-administration when combined with DRV or EVG (GMR range 1.58-2.67; Majeed *et al.*, IWCPAT 2017 and Polina *et al.* 12th Clin Pharm of HIV Therapy Workshop 2011). This finding is important since progestogen overexposure can lead to a number of significant side effects, as described above, that include nausea, weight gain and acne amongst others and that may impact tolerability and adherence.[320]

This study showed that EE C_{24} decreased by 25% with ATV/COBI compared to a 37% decrease reported with ATV/RTV. Moreover, no clinically significant changes were found in C_{max} or AUC_{0-24} (5% and 1% *increases* respectively), compared to the decreases seen with ATV/RTV (19% and 16% respectively).[338] This may be explained by the fact that, unlike RTV, COBI does not induce UGT1A1 or CYP2C9, both of which are involved in EE clearance.[198] As the EE component of the COCP is mainly responsible for endometrial stability and a significant reduction in C_{24} can lead to breakthrough bleeding potentially impacting adherence to contraception,[319] smaller decreases in PK parameters are likely to optimise tolerability and adherence. Our findings also compared well to data on EE co-administration with DRV/COBI in the Majeed *et al.* study, which lead to a 30% and a 14% *decrease* in EE $AUC_{0-\infty}$ and C_{max} respectively, secondary to DRV-mediated induction of CYP2C9 and CYP2C19.[336, 340] EVG-mediated induction of CYP2C9 and UGT appears to overcome general COBI-mediated enzyme inhibition, leading to a *decrease* in EE PK parameters when it is co-administered with Stribild (GMR range 0.66-0.94; Polina *et al.* 12th Clin Pharm of HIV Therapy Workshop 2011).

As previously described, EE concentrations *increase* with unboosted ATV (AUC, C_{max} and C_{min} 48%, 15% and 91%, respectively), as do progestogen concentrations, through ATV mediated CYP3A4 and UGT1A1 inhibition.[339] Yet, when co-administered with ATV/COBI in this study, EE AUC_{0-24}, EE C_{max}, LNG AUC_{0-24} and LNG C_{24} did not increase, whilst EE C_{24} and LNG C_{max} even *decreased*. This is despite the lack of known COBI related enzyme or transporter induction (*in-vitro*) to counteract ATV and COBI-mediated enzyme inhibition. This highlights that COBI's *in-vivo* metabolic

effects may not yet be fully elucidated and warrant further investigation.

Of note, EE C_{max} levels were consistently lower in this study (36-46 pg/mL regardless of the study phase), than in a majority of other EE PK studies (mean levels 80 pg/mL following a 30ug EE dose).[350] This could be explained by the sampling schedule used - EE t_{max} is usually reached at an average of 1.5 hours; it is therefore possible that the maximum concentration reached for some subjects was missed by sampling 1- and 2-hours post-dose. Additionally, levels of EE have been shown to steadily increase within the first treatment cycle,[341] therefore concentrations measured at the end of the cycle (day 21) may be higher than mid-cycle (day 14), which is when subjects in this study underwent intensive PK sampling. It may also be higher again in subsequent cycles. This is however offset by the study design, which allowed for the *intra-individual* effect of co-administration to be measured. LNG PK parameters in this study were comparable to previously published data.[320, 323]

ATV and COBI concentrations were slightly reduced during the co-administration period (**table 5.2**). For ATV, the changes in both groups combined were small and within the no-effect boundary (GMR C_{max} was 0.79 and GMR C_{24} 0.89). Interestingly, there was a significant decrease in ATV/COBI C_{max} and AUC_{0-24} when combined with EE/LNG (GMR 0.64 and 0.68 respectively) in group 1 subjects only (i.e. those who received Microgynon30 alone for 21 days prior to combining with ATV/COBI) whereas no interaction was present with the group 2 subjects. Whilst this could be interpreted as being due to enzyme induction by EE, it is an observation and it is not possible to come to any conclusions based on the very small number of subjects in a sub-group (n=4); C_{24} remained unchanged. Importantly, ATV concentrations in both groups remained reassuringly above the *in-vivo* suggested minimum effective

concentration for wild type HIV (MEC = 150 ng/mL).[335]

The target recruitment was not reached and five/11 participants (45%) withdrew from the study secondary to side effects, 3 of which disclosed information on the reason (rash, deranged liver function tests and gastrointestinal symptoms). This is a higher dropout rate than seen in Majeed *et al.* (22%), although participants in that study had only received one dose of COCP. Whilst high, it is in keeping with the combined published discontinuation rates secondary to any AE for boosted atazanavir (15%) and the COCP (29%).[349, 351] Both are drugs with relatively common side effects in the initial period and it is important that patients are counseled accordingly.

There are limitations to this study. The subjects were HIV negative healthy volunteers. As such, PK or pharmacodynamic comparisons with HIV positive women must be made cautiously and the practical implications of these PK observations are unknown. Clinical outcome data are required in large cohorts of HIV infected participants, and studies investigating pharmacodynamic endpoints (such as failure of viral suppression, HIV-related clinical disease progression or unintended pregnancy) are needed in order to draw definite conclusions on how likely a contraceptive or an ARV is to fail in the context of co-prescription. It is also important to remember that efficacy rates of user dependent contraception differ between perfect use (as seen in a clinical study) and real-life use. The fact that this study involved healthy volunteers may also explain the low number of participants completing the study. In real-life settings, mild side effects associated with the initiation of COCP and/or of ARVs do not normally persist beyond three to six months, at which point an alternative would usually be offered. The option to persist and reassess was not available in the setting of the study.

Nonetheless, this study provides the first steady state PK data on EE/LNG co-administered with ATV/COBI, demonstrating minimal changes in LNG concentrations and a smaller decrease in EE than seen with ATV/RTV. Whilst preliminary, these data are important in informing physicians, who need to discuss and choose safe and reliable contraception with their female patients living with HIV. Whether this minor difference between ATV/RTV and ATV/COBI will be clinically significant warrant further characterisation in future studies.

CHAPTER 6

Genetic Influence Of *ABCG2, UGT1A1* And *NR1I2* On Dolutegravir Plasma Pharmacokinetics

CITATION

Emilie ER, Neary M, Else L, Khoo S, Moyle G, Carr DF, Wang X, Mcclure M, Boffito M, Owen A. Genetic influence of *ABCG2, UGT1A1* and *NR1I2* on dolutegravir plasma pharmacokinetics, *Journal of Antimicrobial Chemotherapy*. https://doi.org/10.1093/jac/dkz558

CONTENT

6.1. INTRODUCTION

DTG is now a preferred agent in major guidelines and a drug of choice for many HIV healthcare providers worldwide.[352] It has replaced EFV as the preferred first-line agent in the WHO ARV guidelines and has been recommended by PEPFAR (Emergency Programme on AIDS Research) for rapid introduction in its key target countries, meaning that it is a major player in the worldwide ARV scale up.[65] Despite a signal raised for a possible increased risk of NTD in women who conceive on DTG (0.3% *vs* 0.12% on other ARVs),[167] the WHO still recommends DTG in women who do not plan to conceive, provided that those of childbearing age are well informed and have access to reliable contraception, until further data is available.[63-65, 164] Overall, up to 60 LMICs have adopted DTG into their national treatment guidelines and it is estimated that 15 million people could be taking DTG by 2025, which stresses the importance of understanding how its pharmacology behaves in diverse and wide-ranging populations.[7, 65]

The real-life rates of DTG-related AEs and discontinuation rates secondary to AEs, particularly NP-AEs, have been discussed in details in **chapters 1 and 2**. A number of cohort studies have suggested an association between DTG PK and neurotoxicity, although this remains to be confirmed.[149-151] Other risk factors such as age, gender, evening dosing and ABC co-administration, have also been suggested in some cohorts, whilst being altogether disproved in others.[129-135, 146] More recently, reports of drug associated weight gain have emerged with InSTIs, particularly DTG and particularly in women and persons of black origin, raising concerns around potential metabolic sequelae.[140-144] Despite TAF being identified as a co-factor and suggestions of possible

DTG-mediated disruptions of adiponectin and/or melanocortin receptor 4 (MCR4) pathways, the aetiology remains poorly understood.[145, 353]

Overall, whilst a relation with DTG C_{min} is suggested, mechanisms of DTG-related AEs, particularly neurotoxicity, are likely to be more complex than a simple linear or threshold-defined PK relationship and may relate to a combination of factors that also include pharmacogenetic, immune and/or functional predispositions.[241, 354]

The PK and PD properties of DTG have already been extensively described in the literature and are discussed in **chapters 1 and 2** of this thesis.[57] To summarise, DTG is primarily metabolised via the phase II enzyme UGT1A1 and, to a minor extent, by phase I CYP3A4 (~ 15%; and UGT1A3, UGT1A9 in vitro), whilst being a substrate for the efflux transporters BCRP and P-gp, which are found on gastrointestinal epithelial cells, liver cells and on endothelial cells within the blood brain barrier.[172] DTG displays no significant CYP enzyme inhibition or induction and thus is a minor DDI perpetrator.[76] Notably, it has a long PK tail (**chapter 3**) and its PK inter-individual variability was moderate in pre-licencing trials (CV%, 24-26%) although greater in subsequent studies (CV% up to 85%).[354-356]

PK data have also become available in populations underrepresented in clinical trials.[57] For instance, in **chapter 2,** a 25% higher C_{max} was demonstrated in PLWH aged 60 years and over and a 25-50% lower exposure (AUC) and C_{min} have been described in women in the 3rd trimester of pregnancy.[65, 160, 354] The DTG SPC reports no ethnicity or gender differences in DTG exposure[235] but this remains to be confirmed more specifically in large, controlled and diverse populations.[76]

Pharmacogenetics data for DTG to date are relatively limited. Chen *et al.,* in a pooled analysis of pharmacogenomics samples collected from pre-licencing clinical studies in healthy subjects, found a 34% lower CL/F, 31% higher AUC_{0-t} and 22% higher C_{max} in homozygous carriers of *UGT1A1* (rs8175347) poor function variants (**28/*37/*6*) compared with subjects with normal enzyme activity (**1/*1* and **1/*36*).[230] This data is referred to in the DTG SPC. Furthermore, in smaller studies, Yagura *et al.* found an association between *UGT1A1*6* (rs4148323) and *UGT1A1*28* (rs8175347) variants and higher C_{min}, whilst Tsuchiya *et al.* reported that DTG C_{max} was significantly higher (50%) in individuals homozygous for *ABCG2 c.*421C>A (rs2231142), which may be particularly important if supratherapeutic DTG concentrations are shown to correlate with DTG side-effects.[148-150, 357] At the time of writing, there were no other published studies investigating the impact of polymorphisms on DTG pharmacokinetics. Of note, however, Borghetti *et al.* recently reported an association between a variant within the encoding gene for OCT2 (*SLC22A2*) and sub-clinical neuropsychiatric pharmacodynamic measurements in a European cohort.[232] DTG inhibits OCT2 but is not a substrate; variants would therefore not be expected to impact DTG plasma concentrations.

Common *UGT1A1* polymorphisms were discussed in **chapter 1**. DTG is also a known substrate of the BCRP efflux transporters. *ABCG2 c.*421C>A (rs2231142) is one of the most studied SNPs for the BCRP encoding gene; the variant allele is most common in East Asian populations (29.1%; Caucasians 10%). It is associated with a loss of protein function and a reduction of drug efflux transport leading to increased substrates plasma and cellular concentrations.[150] Of additional interest, *CYP3A4*22* (522-

191C>T; rs35599367) is associated with lower CYP3A4 expression and activity within the liver,[358] as well as increased lopinavir concentrations.[359] The *CYP3A5**3 (6986A>G, rs776746) variant allele, whilst not directly involved in DTG metabolism, is known to be in linkage disequilibrium with *CYP3A4**1B and has been independently associated with higher NVP AUC and reduced ATV clearance.[222, 360, 361] *NR1I2* encodes the PXR nuclear receptor, which regulates the expression and activity of several enzymes, including CYP3A4 and UGT1A1.[222, 362] *NR1I2* c.63396C>T (rs2472677) has been associated with the PK of unboosted atazanavir.[363, 364] Accordingly, the objective of this study was to investigate the role of common *UGT1A1*, *ABCG2, CYP3A and NR1I2* SNPs on plasma DTG concentrations in pooled subjects from four clinical trials investigating the PK of 50mg DTG taken OD. Of note, *ABCB1* SNPs, coding for P-gp, were not selected since there are many known compensatory mechanisms for any potential ABCB1 polymorphism related PK/PD effects.

6.2. METHODS

6.2.1. Clinical study and participant selection

Pooled samples from three Phase I (SSAT061 (NCT02219217), SSAT064 (NCT02509195) and SSAT073 (NCT03094507)) and one Phase III (SSAT066 (NCT02351908)) clinical trials carried out at the St Stephen's AIDS Trust clinical trial unit, London, between 2014 and 2017 were collected and saved for genetic analysis. All Phase I trials were clinical pharmacology repeat-dose studies involving intensive PK assessments. The Phase III trial included a PK sub-study involving a timed DTG C_{24} at steady state. All studies used a 50mg dose of DTG OD, taken as a tablet formulation either alone (healthy volunteers) or co-formulated with

abacavir/lamivudine as part of Triumeq® in the Phase I studies and with emtracitabine/tenofovir (Truvada®) in the Phase III study (HIV infected participants). All clinical studies are registered.

The studies selected for inclusion were conducted in accordance with good clinical practice procedures, all applicable regulatory requirements and the guiding principles of the Declaration of Helsinki. The study protocols for each clinical study were reviewed and approved by the applicable National Research Ethics Service (NRES) committees and MHRA UK. Pharmacogenetic samples were collected under separate written informed consent to the main clinical study and were optional for participants in each study. The respective NRES committees for each study approved the pharmacogenetic sub-study for each trial as part of the main study protocol approval. No individual subject took part in more than one study.

6.2.2. PK sample and data collection

Within each of the Phase I studies, subjects underwent steady state intensive DTG plasma PK determinations, following witnessed drug intake (on study day 14 or 28). Blood samples were collected pre-dose, 1, 2, 3, 4, 8, 12- and 24-hours post-dose. The Phase III study involved a one-off PK sample taken 24-hour post-dose. This was carefully timed, with research staff instructing participants over the phone to take the medication the morning of dosing and with participants attending the clinical research unit the following day to allow for PK sampling exactly 24-hours post-dose, as timed by research staff. Overall, medication adherence was assessed through direct questioning and pill count in all studies. Steady-state plasma concentrations were determined using high-pressure liquid chromatography–tandem mass spectrometry

methods for samples from three of the clinical trials (HPLC-MS/MS; completed at the Bioanalytical Facility, University of Liverpool) or using ultra-performance liquid chromatography coupled with UV detection for one clinical trial (UPLC; Jefferiss Trust Laboratory, Imperial College London). Both are validated and have been previously described in the literature.[247, 273] The lower limits of quantification (LLOQ) were 10 ng/mL and 80 ng/mL, respectively. For concentrations below the assay LLOQ, a value of one-half of the quantification limit was used. Accuracy (percentage bias) was between 92.5% and 96.2% and precision was between 2.6% and 4.1% for the HPLC-MS/MS method, whilst the reported accuracy for the UPLC method was between 90.7% and 97.7%, intra-assay variability was 3.3–6.1%, and inter-assay variability was 4.5–5.7%.[247, 273] The two methods have not been cross-validated as their respective calibration ranges vary widely. The calculated PK parameters for plasma DTG in the three phase I studies were C_{24}, C_{max}, AUC_{0-24} and half-life ($t_{1/2}$). For the phase III study, only C_{24} was determined. PK parameters were calculated using actual blood sampling time and non-compartmental analysis techniques (WinNonlin Phoenix; version 6.1 or above; Pharsight, Mountain View, CA).

6.2.3. Pharmacogenetics sampling, DNA extraction and genotyping

Venous blood was collected at baseline, from subjects consenting to pharmacogenetics research, into an EDTA vacutainer. Samples were then shipped on dry ice to the Pharmacology Research Laboratories at the University of Liverpool, UK, and stored at -80°C. Genomic DNA was extracted from whole blood using a spin-column based kit according to the manufacturer's protocol (E.Z.N.A Blood DNA Mini Kit; Omega bio-tek; Norcross, GA). Extracted DNA was quantified using NanoDrop (ThermoFisher Scientific, Wilmington, DE). Genotyping was completed using real

time allelic discrimination polymerase chain reaction (PCR) assays on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Hercules, CA). The PCR protocol followed denaturation at 958°C for 10 min, followed by 50 cycles of amplification at 928°C for 15 sec and annealing at 608°C for 1 min 30 sec.

Taqman Genotyping Master mix and assays were purchased from Thermofisher Scientific (Life Technologies Ltd, Paisley, Renfrewshire, UK) and were as follows: *CYP3A4*22* c.522-191C>T (rs35599367, catalogue number C_59013445_10), *CYP3A5*3* c.6986A>G (rs776746, catalogue number C_26201809_30), *ABCG2* c.421C>A (rs2231142, catalogue number C_15854163_70), *ABCG2* c.34G>A (rs2231137, made to order), *NR1I2* c.63396C>T (rs2472677, catalogue number C_26079845_10), *NR1I2* c.44477A>G (rs1523130, catalogue number C_9152783_20) and *UGT1A1*6* c.211G>A (rs4148323, catalogue number C_559715_20). Opticon Monitor V3.1 software (Bio-Rad Laboratories) was used to obtain allelic discrimination plots and identify genotypes. The *UGT1A1* promoter region (**1, *28, *36* and **37*) was genotyped using an Agena MassArray iPLEX assay.

6.2.4. Covariates

Subjects age, gender, height, weight, ethnicity, HIV status and accompanying drug to DTG were extracted from each study. Covariates were then included in the univariate and multivariate linear regressions described below.

6.2.5. Statistical analysis

In order to determine assay performance, genotypes for each marker were evaluated for compliance with Hardy–Weinberg equilibrium ($p > 0.05$) using validated and previously outlined methods.[365] Allele frequencies were also compared to published

and publicly available British and European allele frequencies.[242]

Genotypes were coded for regression analyses as 0 for the homozygous common allele, 1 for the heterozygous and 2 for the homozygous variant allele. For SNPs displaying a dominant or recessive allele effect, coding was dichotomized and weighted appropriately (e.g. if using a recessive genotypic test model, the homozygote common variant and the heterozygote allele were grouped into a single category and were coded as 0 whilst the homozygote variant was coded as 2).[366] The SNPs selected for the study were analyzed individually. SNPs found to correlate with any PK parameter were then also combined in pairs to create scoring algorithms consisting of the sum of each genotype code.

Categorical variables were described using relative frequencies, while continuous variables were described using medians and interquartile ranges (IQR). Drug PK parameters were described using GM (95% CI). Inter-individual variability in PK parameters was expressed as CV%. The Shapiro–Wilk test was applied to test continuous variables for normality, with $p < 0.05$ considered statistically significant; variables were Log_{10} transformed if the normality test failed.

Associations between participant covariate characteristics or genotypes and DTG concentrations were determined through univariate and multivariate linear regressions. Univariate linear regression with a p value of <0.2 were carried through to multivariate linear regression analysis where a p value of <0.05 was classed as statistically significant. Results were checked with the Benjmini-Hochberg procedure to account for multiple comparisons, using a false positivity rate (Q value) of 10%. All statistical

analyses were carried out using IBM SPSS Statistics v.22 (IBM, Armonk, NY). Charts were produced using GraphPad Prism 8 (GraphPad Software, La Jolla, CA).

6.3. RESULTS

6.3.1. Participants

One hundred participants attended the baseline visit of one of the four clinical trials. Two subjects declined participation to a genomic sub-study and 5 withdrew from their trial before PK data were collected. Ninety-three subjects with paired pharmacogenetic and PK data were pooled for analysis (57 HIV-infected and 36 healthy volunteers; 67 men and 26 women). Subject characteristics and genotype frequencies are summarised in **table 6.1**. The median (IQR) age and weight were 51 years (35–64 years) and 77 kg (67-84); 71% self-described as Caucasian and 17% as Black African or Black Caribbean.

6.3.2. DTG pharmacokinetics

76 participants provided intensive PK data collected over 24 hours and 17 provided a single PK sample 24 hours post-dose (C_{24}). 53 samples were analysed using HPLC-MS/MS and 40 using UPLC. All participants received 50mg OD DTG, taken in the morning of the intensive PK day or the morning before the one-off PK measurement. DTG GM (95% CI) for C_{max}, $AUC_{0\text{-}24,}$ C_{24} and $t_{1/2}$ were 3974 ng/mL (3864 - 4357), 51846 ng*h/mL (48607- 55085), 1182 ng/mL (994 - 1371) and 13.0 hours (12.1 - 14.0). DTG PK parameters are summarised by SNP in **table 6.2**.

6.3.3. Genotypes overview

All SNPs were in Hardy–Weinberg equilibrium, except for *CYP3A5*3* c.6986A>G

(rs776746; X_2 = 33.36; p=0.001) and *CYP3A4*22* c.522-191C>T (rs35599367; X_2 = 33.13; p=0.001), which may compromise their interpretation (although both still mirrored European genotype distributions). Genomic data were missing in 1 case for *CYP3A5*3*, in 1 case for *UGT1A1*6* and in 9 cases for *UGT1A1*28* due to assay failure. Univariate and multivariate regression analyses with significant associations for DTG PK parameters are presented in **table 6.3** whilst figure **6.1** shows scatter plots for each statistically significant genotype, plotting DTG PK data (GM) versus genotype for each SNP. The totality of the regression results can be found in **table 6.4.**

6.3.4. Covariates

Weight and Log10 height were associated with lower DTG Log10 C_{max} (β=-1.649 p=0.012 and β=-0.003; p=0.009, respectively) whilst DTG administration with ABC/3TC was associated with a higher DTG Log10 C_{max} than intake alone (GM C_{max} (95% CI) 4246 (3872-4620) *vs* 3692 (3414-3971) ng/mL, p=0.001). TDF/FTC co-administration with DTG was associated with a higher DTG Log10 C_{24} than administration alone or with ABC/3TC (GM C_{24} (95% CI): 1791 (975-2607) *vs* 1106 (976-1236) & 1052 (876-1228) ng/mL, respectively; β=0.069; p=0.034). Finally, higher weight was also associated with lower DTG Log10 AUC_{0-24} (β=-0.002; p=0.02), with an 8-10% lower GM AUC_{0-24} for every 10kg increase in weight bracket between 40 and 80kg.

6.3.5. *ABCG2* c.421C>A (rs2231142)

After multivariate analysis, *ABCG2 c.*421C>A (rs2231142) was independently associated with a 28% higher DTG C_{max} (β=0.053, p=0.047) in the homozygous variant carriers. GM C_{max} (95% CI) was 3893 (3774-4240), 4346 (3629-5531) and 4994 (single value) ng/mL in the CC, CA and AA genotype groups, respectively.

	Total N		
	93		
	Median (IQR)		
Age (years)	51 (36-64)		
Weight (kg)	77.6 (67-84.4)		
Height (cm)	173 (168-177)		
ARV Regimen	**N (%)**		
• **ABC/3TC/DTG**	40 (43)		
• **TFV/FTC + DTG**	17 (18)		
• **DTG alone**	36 (39)		
Ethnicity	**N (%)**		
• **Caucasian**	70 (75)		
• **Black**	16 (17)		
• **Asian**	3 (3)		
• **Mixed race**	1 (1)		
• **Other**	3 (3)		
Female gender	26 (28)		
	PK parameters GM (95% CI) – IQR		
DTG GM C_{max} (ng/mL)	3974 (3864 – 4357) – IQR 3462-4611		
DTG GM AUC_{0-24} (hr*ng/mL)	51846 (48607- 55085) – IQR 53190-57191		
DTG GM C_{24} (ng/mL)	1182 (994 – 1371)) – IQR 873-1612		
DTG GM $t_{1/2}$ (hrs)	13 (12.0- 14.0) – IQR 11.0-15.3		
	Genotypic frequencies %		
UGT1A1**28 (rs8175347)*	**Extensive metaboliser**	**Intermediate metaboliser**	**Poor metaboliser**
	46	43	11
***UGT1A1**6 c.211G>A (rs4148323)**	**Extensive metaboliser**	**Intermediate metaboliser**	**Poor metaboliser**
	37	63	0
***CYP3A4**22 G>A (rs35599367)**	**GG**	**GA**	**AA**
	88	6	5
***CYP3A5**3 C>T (rs776746)**	**CC**	**TC**	**TT**
	76	12	12
***ABCG2* 421C>A (rs2231142)**	**CC**	**CA**	**AA**
	82	17	1
***ABCG2* 34C>T (rs2231137)**	**CC**	**CT**	**TT**
	83	17	0
***NR1I2* 63396C>T (rs2472677)**	**CC**	**CT**	**TT**
	17	42	41
***NR1I2* 44477T>C (rs1523130)**	**TT**	**CT**	**CC**
	19	39	42

Table 6.1: Characteristics of participant population are shown as medians (interquartile range) or count (N), percentage of population (%). PK values are shown as geometric means (GM) (95% Confidence Interval, 95% CI). CV% = percentage coefficient variation. **Clinical Pharmacogenetics Implementation Consortium (CPIC) classification for UGT1A1 genotype-predicted phenotypic function: extensive metabolisers (*1/*1; *1/*36; *36/*36), intermediate metabolisers (*1/*28; *1/*37; *36/*28; *36/*37; *1/*6) and poor metabolisers (*28/*28; *28/*37; *37/*37; *6/*6

6.3.6. *NR1I2* c.63396C>T (rs2472677)

NR1I2 c.63396C>T (rs2472677) was associated with higher DTG Log10 C_{max} (β=0.032; *p*=0.033) and higher DTG Log10 AUC_{0-24}, (β= 0.042; *p*=0.029). GM C_{max} (95% CI) was 3445 (3176-3822), 3938 (3705-4480) and 4278 (3992-4817) ng/mL and GM AUC_{0-24} (95% CI) was 42750 (38002-52263), 54138 (50998-61344) and 54170 (51019-60413) ng*h/mL in the CC, CT and CC genotype groups, respectively. This represents a 24% difference in C_{max} and a 27% difference in AUC_{0-24} between homozygote groups.

6.3.7. *UGT1A1**28 (rs8175347)

The *UGT1A1**28 variant allele displayed a recessive allele effect (**figure 6.1**). Coding was therefore dichotomized and weighted appropriately (extensive and intermediate metabolisers were grouped as a single category coded as 0 and poor metabolisers were coded as 2). The *UGT1A1**28 poor metaboliser genotype was independently associated with higher DTG Log10 AUC_{0-24} (β=0.042; p=0.02). GM AUC_{0-24} (95% CI) were 52639 (47956-57321), 51818 (46866-56771) and 66281 (57162-75401) ng*h/mL for the extensive, intermediate and poor metaboliser genotypes respectively (27% difference between homozygote groups). When *UGT1A1**28 was combined with *UGT1A1**6, genotypic scores ≥three/4 were associated with a 36% increase in DTG AUC_{0-24} and a 44% increase in DTG C_{24} (β=0.041; p=0.023 and β=0.042; p=0.009 respectively). GM AUC_{0-24} (95% CI) was 48500 (43417-53583) ng*h/mL in participants who scored 0 and 66085 (54917-77253) ng*h/mL in those who scored three/4 (no individual scored four/4). GM C_{24} (95% CI) was 1109 (885-1334) and 1594 (1247-1941) ng/mL respectively.

6.3.8. Composite Scores

6.3.8.1. *UGT1A1*28 + *NR1I2* c.63396C>T

Participants carrying the homozygous variant alleles for both *NR1I2* c.63396C>T (rs2472677) and *UGT1A1*28* displayed a statistically significant 79% higher AUC_{0-24} (β=0.42, p=0.005). GM AUC_{0-24} (95% CI) in those who carried the common allele for both genotypes was 42306 (36990-52278) *vs* 75807 (69714-82166) ng*h/mL in those who carried the variant allele for both. This was the largest magnitude of SNP effects seen in this study. Variability in the two groups as reflected by IQR was 41921-47692 and 73180-74542 ng*h/mL respectively. There was also a significant 47% increase in C_{max} and a 78% increase in GM C_{24}. The latter was not statistically significant (p=0.436).

6.3.8.2. *ABCG2* c.421C>A (rs2231142) and *NR1I2* c.63396C>T

When combined into a scoring algorithm, a statistically significant 43% higher C_{max} and 39% higher AUC_{0-24} were seen in participants who scored ≥three/4 relative to participants who scored 0 (β=0.038; p=0.002 and β=0.038; p=0.002 respectively). GM C_{max} (95% CI) were 3450 (3102-3799) *vs* 4924 (3555-6293) ng/mL and GM AUC_{0-24} (95% CI) were 42768 (35078-50457) *vs* 59335 (48362-70308) ng*h/mL in the two groups respectively. Only one person scored four/4 and they were categorised with those who scored three/4. A genotypic score-dose effect was seen.

There were no significant differences in genotypic distribution for *ABCG2*, *UGT1A1* and *NR1I2* between groups analysed with either HPLC-MS/MS or UPLC for PK.

Remaining genotypes

No clinically significant association was found with the remaining genotypes studied.

	Allele	C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)	C_{24} (ng/mL)	$t_{1/2}$ (hrs)
*CYP3A4*22 (rs35599367)*	GG	3942 (3816- 4356)	50689 (49364- 56391)	1153 (1133-1548)	12.96 (12.52-14.56)
	GA	3820 (3761-4284)	57590 (42549- 63889)	1426 (647-2124)	12.67 (10.11-17.13)
	AA	4571 (3745-5581)	64494 (57914-71837)	1430 (1253-1634)	14.20 (11.70-17.26)
*CYP3A5*3* (rs776746)	TT	4011 (3526-4722)	57328 (51748- 64881)	1295 (1167-1475)	13.40 (11.92-15.37)
	TC	4333 (3544-5545)	57339 (51437-65205)	1313 (1014-1838)	13.48 (11.87-15.66)
	CC	3898 (3753-4294)	49972 (48290-56523)	1159 (1129-1611)	13.03 (12.47-14.83)
ABCG2 c.421C>A (rs2231142)	CC	3893 (3774-4240)	51179 (49757-56852)	1037 (1156-1578)	13.15 (12.72-14.51)
	CA	4346 (3629-5531)	54765 (48083-65483)	1122 (882-1801)	12.29 (9.75-16.81)
	AA	4994 (-)	60762 (-)	1310 (-)	15.55 (-)
ABCG2 c.34C>T (rs2231137)	CC	3936 (3812-4310)	51381 (49992-57307)	1021 (1148-1585)	12.90 (12.43-14.57)
	CT	4163 (3543-5153)	54158 (48523-62306)	1230 (1020-1662)	13.75 (12.35-15.63)
	TT	-	-	-	-
NRI1/2 c.63396C>T (rs2472677)	CC	3445 (3176-3822)	42750 (38002-52263)	1094 (910-1539)	11.93 (10.63-13.95)
	CT	3938 (3705-4480)	54138 (50998-61344)	946 (1171-1632)	13.05 (12.20-14.98)
	TT	4278 (3992-4817)	54170 (51019-60413)	1165 (1002-1757)	13.51 (12.50-15.72)
NRI1/2 c.44477A>G (rs1523130)	TT	3830 (3581-4191)	53505 (49786-59019)	1158 (1047-1387)	12.68 (11.51 -14.62)
	CT	3933 (3636-4537)	50105 (46431-57750)	1031 (1049-1904)	13.14 (11.85 -15.79)
	CC	4087 (3826-4677)	52298 (49481-60752)	1031 (1119-1528)	13.15 (12.34 -15.03)
*UGT1A*28* (rs8175347)	Extensive	4152 (3731-4573)	52639 (47956-57321))	1115 (934-1297)	12.43 (11.22 -13.65)
	Intermediate	4015 (3665-4364)	51818 (48966-58871)	1212 (973-1452)	13.57 (11.82 -15.32)
	Poor	4512 (3733-5291)	66281 (57162-75401)	1600 (1294-1906)	14.85 (11.91 -17.79)
*UGT1A1*6* (rs4148323)	GG	4205 (3946-4767)	51735 (48953-597470	1085 (1015-1399)	13.00 (11.76-14.24)
	GA	3797 (3622-4201)	51935 (49667-57553)	1221 (1147-1690)	13.06 (11.73-14.39)
	AA	-	-	-	-

Combined *UGT1A1*6* and **28* scores	0	3966 (3639-4294)	48500 (43417-53583)	1109 (885-1334)	12.72 (11.93-14.40)
	1	4189 (3765-4613)	55837 (51521-60152)	1208 (1008-1407)	13.53 (12.50-15.89)
	2	-	-	-	
	3	4678 (3812-5545)-	66085 (54917-77253)	1594 (1247-1941)	13.65 (11.21-16.36)
Combined *UGT1A1*28* and *NR1I2* c.63396C>T scores	0	3626 (3361-3950)	42306 (34990-52278)	1097 (868-1619)	10.97 (9.64-12.54)
	1	4151 (3855-4737)	57502 (53574-64634)	1366 (1253-1801)	13.76 (12.23-15.29)
	2	4207 (3887-4774)	52718 (49568-58223)	1027 (954-1230)	13.17 (11.51-14.83)
	3	3844 (3238-4477)	53789 (53659-53918)	1132 (973-1304)	16.88 (12.15-21.60)
	4	5333 (4570-6159)	75807 (69714-82166)	1958 (1703-2253)	x
Combined *ABCG2* c.421C>A and *NR1I2* c.63396C>T scores	0	3450 (3102-3799)	42768 (35078-50457)	1124 (795-1453)	11.98 (10.19-13.78)
	1	3915 (3528-4301)	53948 (48733-59162)	1204 (980-1429)	13.38 (11.95-14.80)
	2	4077 (3763-4392)	52720 (47997-57443)	1217 (217-791)	13.05 (11.73-14.36)
	3	4924 (3555-6293)	59335 (48362-70308)	1067 (740-1394)	13.72 (8.15-19.29)

Table 6.2: Dolutegravir (DTG) pharmacokinetic parameters shown as Geometric Means (GM) 95% Confidence Interval (95% CI), summarised by single or combined genotype. NB: *UGT1A1*28* was coded as binary

Log_{10} C_{max}	**Univariate Linear Regressions**			**Multivariate Linear Regressions**		
	***p* value**	**β value (95% CI)**	**r^2**	***p* value**	**β value (95% CI)**	**r^2**
Log_{10}Height (Log_{10}cm)	0.008	-1.716	0.092	**0.012**	-1.649	0.394
Weight (kg)	0.000	-0.004	0.175	**0.009**	-0.003	0.394
Accompanying Drug	0.019	0.061	0.072	**0.001**	0.074	0.394
*UGT1A1*6* (rs4148323)	0.091	-0.044	0.038	0.355	0.039	0.402
***ABCG2* c.421C>A (rs2231142)**	0.111	0.050	0.034	**0.047**	0.053	0.394
***NR1I2* c.63396C>T (rs2472677)**	0.010	0.045	0.086	**0.033**	0.032	0.394
Combined *NRI1/2* and UGT1A1*28 scores	0.030	0.029	0.071	**0.023**	0.026	0.311

Combined *ABCG2* and *NRI1/2* scores	0.011	0.057	0.291	**0.005**	0.054	0.377
Log_{10} C_{24}	**Univariate Linear Regressions**			**Multivariate Linear Regressions**		
	***p* value**	**β value (95% CI)**	**r^2**	***p* value**	**β value (95% CI)**	**r^2**
Log_{10} Age (Log_{10}years)	0.125	-0.236	0.026	**0.029**	-0.310	0.104
Accompanying drug	0.008	0.084	0.076	0.114	0.048	0.133
***UGT1A1*28* (rs8175347)**	0.045	0.070	0.049	**0.083**	0.059	0.140
Combined *UGT1A1*6* and **28* scores	0.009	0.067	0.082	**0.009**	0.067	0.082
AUC_{0-24}	**Univariate Linear Regressions**			**Multivariate Linear Regressions**		
	***p* value**	**β value (95% CI)**	**r^2**	***p* value**	**β value (95% CI)**	**r^2**
Log_{10}Height (Log_{10}cm)	0.011	-1.866	0.066	0.323	-0.871	0.282
Weight (kg)	0.017	0.003	0.075	**0.03**	-0.002	0.228
Ethnicity	0.044	0.036	0.054	0.143	0.025	0.256
*CYP3A4**22 (rs35599367)	0.059	0.053	0.047	0.295	0.027	0.270
***CYP3A5**3 (rs776746)**	**0.097**	**-0.034**	**0.037**	**0.033**	**-0.040**	**0.228**
***NR1I2* c.63396C>T (rs2472677)**	0.033	0.043	0.060	**0.029**	0.042	0.228
***UGT1A1*28* (rs8175347)**	0.020	0.058	0.060	**0.020**	0.116	0.228
Combined *UGT1A1*6* and **28* scores	0.075	0.046	0.048	**0.041**	0.050	0.231
Combined *UGT1A1*28* and *NRI1/2* scores	0.011	0.039	0.095	**0.002**	0.048	0.025
$t_{1/2}$	**Univariate Linear Regressions**			**Multivariate Linear Regressions**		
	***p* value**	**β value (95% CI)**	**r^2**	***p* value**	**β value (95% CI)**	**r^2**
Ethnicity	**0.057**	0.031	0.058			

Table 6.3: Significant results from univariate ($p < 0.2$) and multivariate ($p < 0.5$) linear regression analysis per PK parameter (significant SNP associations are boxed in bold)

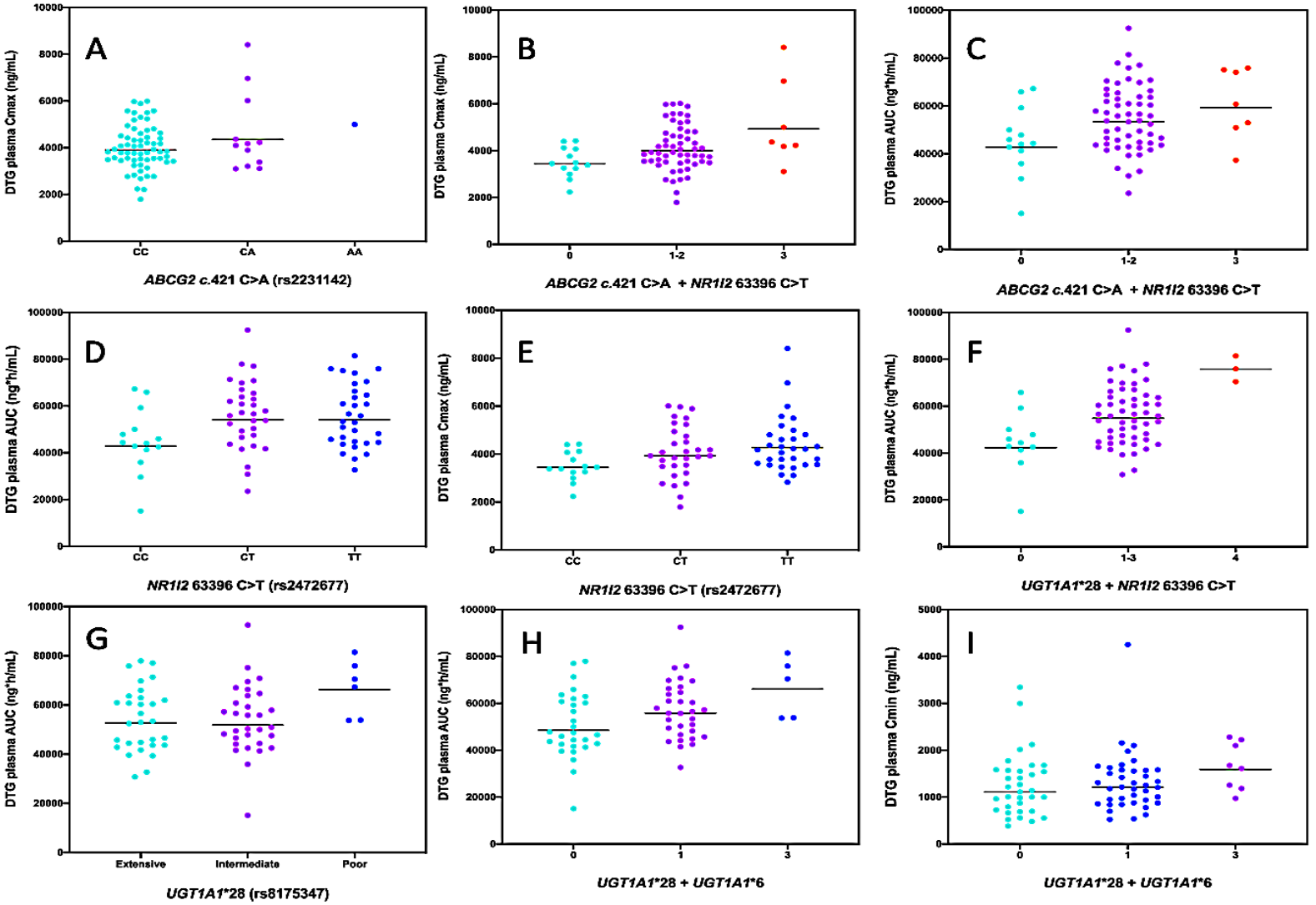


Figure 6.1: Scatter plots of statistically significant relationships between genotypes and DTG plasma PK parameters (Geometric Means)

Log10 C_{max}	Univariate Linear Regressions			Multivariate Linear Regressions			Effect size
	p value	β value	r^2	*p* value	β value	r^2	
Log_{10} Age (Log_{10}years)	0.252	0.104	0.018				
Log_{10}Height (Log_{10}cm)	**0.008**	**-1.716**	**0.092**	**0.012**	**-1.649**	**0.394**	**-**
Weight (kg)	**0.000**	**-0.004**	**0.175**	**0.009**	**-0.003**	**0.394**	**-**
Ethnicity	0.297	0.002	0.015				
Accompanying drug	**0.019**	**0.061**	**0.072**	**0.001**	**0.074**	**0.394**	**-**
*CYP3A4*22* (rs35599367)	0.309	0.025	0.014				
*CYP3A5*3* (rs776746)	0.514	- 0.012	0.006				
***ABCG2* c.421C>A (rs2231142)**	**0.111**	**0.050**	**0.034**	**0.047**	**0.053**	**0.394**	**28%**
ABCG2 c.34C>T (rs2231137)	0.487	0.024	0.007				
***NR1I2* c.63396C>T (rs2472677)**	**0.010**	**0.045**	**0.086**	**0.033**	**0.032**	**0.394**	**24%**
NR1I2 c.44477A>G (rs1523130)	0.384	0.014	0.010				
*UGT1A1*6* (rs4148323)	**0.091**	-0.044	0.038	0.355	0.039	0.402	
*UGT1A1*28* (rs8175347) **	0.332	0.044	0.014				
Combined *NRI1/2* and UGT1A1*28 scores	**0.030**	**0.029**	**0.071**	**0.023**	**0.026**	**0.311**	**47%**
UGT1A1*6 and UGT1A1*28	0.797	0.004	0.001				18%
Combined *ABCG2* c.421C>A and *NRI1/2* c.63396C>T scores	**0.004**	**0.040**	**0.105**	**0.002**	**0.038**	**0.390**	**43%**
Log10 C_{24}	Univariate Linear Regressions			Multivariate Linear Regressions			Effect size
	p value	β value	r^2	*p* value	β value	r^2	
Log_{10} Age (Log_{10}years)	**0.125**	**-0.236**	**0.026**	**0.029**	**-0.310**	**0.104**	**-**
Log_{10}Height (Log_{10}cm)	0.363	1.033	0.009				
Weight (kg)	0.454	-0.001	0.006				
Healthy volunteer	0.697	-0.047	0.011				
Ethnicity simplified coding	0.526	0.004	0.017				
Accompanying drug	**0.008**	0.084	0.076	0.114	0.048	0.133	
*CYP3A4*22* (rs35599367)	0.221	0.057	0.016				
*CYP3A5*3* (rs776746)	0.390	-0.029	0.008				
ABCG2 c.421C>A (rs2231142)	0.766	-0.016	0.001				
ABCG2 c.34C>T (rs2231137)	0.735	0.021	0.001				
NR1I2 c.63396C>T (rs2472677)	0.847	0.006	0.000				
NR1I2 c.44477A>G (rs1523130)	0.922	0.003	0.000				
*UGT1A1*6* (rs4148323)	0.281	0.051	0.013				
*UGT1A1*28* (rs8175347)**	**0.045**	0.070	0.049	0.083	0.059	0.140	38%
Combined ***UGT1A1*6 and *28***	**0.009**	**0.067**	**0.082**	**0.009**	**0.067**	**0.082**	**44%**
Combined *ABCG2* c.421C>A and *NRI1/2* c.63396C>T scores	0.436	0.017	0.008				39%
Combined *NRI1/2* c.63396C>T and *UGT1A1**28 scores	0.436	0.017	0.008				78%
Log10 AUC_{0-24}	Univariate Linear Regressions			Multivariate Linear Regressions			Effect size
	p value	β value	r^2	*p* value	β value	r^2	
Log_{10} Age (Log_{10}years)	0.291	-0.110	0.015				
Log_{10}Height (Log_{10}cm)	**0.011**	-1.866	0.066	0.323	-0.871	0.282	
Weight (kg)	**0.030**	-0.002	0.075	**0.03**	-0.002	0.228	
Healthy volunteer	0.978	0.001	0.000				
Ethnicity simplified coding	**0.044**	0.036	0.054	0.143	0.025	0.256	
Accompanying drug	0.978	-0.001	0.000				
*CYP3A4*22* (rs35599367)	0.059	0.053	0.047	0.295	0.027	0.270	
***CYP3A5*3* (rs776746)**	**0.097**	**-0.034**	**0.037**	**0.033**	**-0.040**	**0.228**	**-12%**

ABCG2 c.421C>A (rs2231142)	0.376	0.032	0.011				
ABCG2 c.34C>T (rs2231137)	0.566	0.023	0.004				
***NR1I2* c.63396C>T (rs2472677)**	**0.033**	**0.043**	**0.060**	**0.029**	**0.042**	**0.228**	**27%**
NR1I2 c.44477A>G (rs1523130)	0.893	-0.003	0.000				
UGT1A1*28* (rs8175347)*	**0.020**	**0.058**	**0.060**	**0.020**	**0.116**	**0.228**	**27%**
*UGT1A1*6* (rs4148323)	0.956	0.002	0.000				
Combined *UGT1A1*6* and **28*	**0.035**	**0.041**	**0.067**	**0.023**	**0.041**	**0.243**	**36%**
Combined *ABCG2* c.421C>A and *NRI1/2* c.63396C>T scores	**0.031**	**0.035**	**0.061**	**0.008**	**0.042**	**0.257**	**43%**
Combined *NRI1/2* c.63396C>T and *UGT1A128 scores**	**0.011**	**0.039**	**0.095**	**0.002**	**0.048**	**0.225**	**78%**
$t_{1/2}$	Univariate Linear Regressions			Multivariate Linear Regressions			Effect size
	p value	β value	r^2	p value	β value	r^2	
Log_{10} Age (Log_{10}years)	0.506	-0.069	0.006				
Log_{10}Height (Log_{10}cm)	0.495	0.529	0.007				
Weight (kg)	0.484	0.001	0.007				
Healthy volunteer	0.637	0.014	0.003				
Ethnicity	0.437	0.008	0.003				
Accompanying drug	0.637	-0.014	0.003				
*CYP3A4*22* (rs35599367)	0.561	0.016	0.005				
*CYP3A5*3* (rs776746)	0.719	-0.008	0.002				
ABCG2 c.421C>A (rs2231142)	0.744	-0.011	0.002				
ABCG2 c.34C>T (rs2231137)	0.487	0.028	0.007				
NR1I2 c.63396C>T (rs2472677)	0.212	0.025	0.022				
NR1I2 c.44477A>G (rs1523130)	0.707	0.007	0.002				
*UGT1A1*28* (rs8175347)**	0.274	0.058	0.020				
*UGT1A1*6* (rs4148323)	0.950	0.002	0.000				
Combined *UGT1A1*6* and **28*	0.302	0.021	0.017				
Combined *ABCG2* c.421C>A and *NRI1/2* c.63396C>T scores	0.391	0.014	0.011				
Combined *NRI1/2* c.63396C>T and *UGT1A1**28 scores	**0.057**	0.031	0.058				

Table 6.4 Univariate and Multivariate regressions – complete analysis. Results in bold = above cut off; ***UGT1A1**28 dichotomised throughout, using a recessive genotypic test model

6.4. DISCUSSION

The impact on DTG PK parameters of key common SNPs coding for the four main enzymes and transporters involved in its disposition were investigated.[172] For the first time, to the best of our knowledge, these were brought together in a multivariate analysis model, which also controlled for important demographic covariates and were combined into scoring algorithms.

A statistically significant increase in DTG exposure was found in carriers of the *UGT1A1**28 (rs8175347) poor metaboliser genotype (28%). Our results are in keeping with findings from the Chen *et al.* study albeit with a smaller effect size (28% *vs* 46%).[230] As previously discussed, Yagura *et al.* reported an association between the **28* heterozygote status (intermediate metaboliser) and an increase in C_{min} but not for the homozygote (poor metaboliser) genotype, which the authors related to lack of statistical power.[148] Contrastingly, our study demonstrated higher DTG PK parameters in the *UGT1A1**28 poor metaboliser group and not the intermediate group, consistent with a recessive genotype model. Whilst this may also relate to a potential lack of statistical power, other studies have reported a lack of PK or PD effect with the *UGT1A1**28 intermediate metaboliser genotype with drugs such as irinotecan or raloxifene.[367, 368] Of note, the impact of *UGT1A1**28 alone on DTG concentrations seemed modest compared to that seen for raltegravir concentrations, where C_{12} was 110% higher in individuals carrying *UGT1A1**28 poor metaboliser genotype.[369] However, when *UGT1A1**28 and *NR1I2* c.63396C>T were combined in our study, a 79% higher AUC_{0-24} and a 47% higher C_{max}, were seen in those with a maximum score (C_{24} was 78% higher but this was not statistically significant). Additionally, when *UGT1A1**28 and *UGT1A1**6 were combined, genotypic scores ≥three/4 were associated with a 36% higher DTG AUC_{0-24} and a 44% higher DTG C_{24}. Overall, this indicates that genomic biomarkers of DTG plasma exposure may be better based on carefully defined sets of SNPs or scoring algorithms rather than on single SNP characterisation.[228, 370-373]

A statistically significant, but moderate, 28% higher DTG C_{max} was found in subjects carrying the *ABCG2* c.421C>A *(*rs2231142) homozygous variant genotype (AA)

compared to those homozygous for the common allele (CC). This therefore confirms, results previously reported by Tsuchiya *et al.* but in a very different population (75% Caucasian and 17% Black participants (n=76) *vs* 100% Japanese participants (n=42) respectively).[357] The higher DTG C_{max} may reflect a decrease in first pass metabolism, for instance through reduced expression of efflux BRCP transporters in intestinal epithelial cells leading to increased absorption and/or through reduced hepatic clearance.[357, 374] Higher exposures to sunitinib, rosuvastatin and atorvastatin have similarly been described in individuals carrying the *ABCG2* c.421C>A (rs2231142) variant.[375, 376] Interestingly, when *ABCG2* c.421C>A was combined with *NR1I2* c.63396C>T (rs2472677), participants homozygous for the variant in both genes showed a significant 43% increase in DTG C_{max}, once again suggesting a composite of SNPs may represent a more useful genomic biomarker of DTG PK parameters.

The *NR1I2* c.63396C>T (rs2472677) variant was independently associated with higher C_{max} (24%) and $AUC_{0\text{-}24}$ (27%). This is converse to the effects seen with unboosted atazanavir[363, 364] and is surprising since the TT genotype is thought to be associated with higher basal expression of the nuclear receptor PXR, which in turn would be expected to result in higher UGT1A1, BCRP and CYP3A4 expression and lower DTG concentrations.[212, 222] Therefore, this observation should be interpreted with caution and needs to be confirmed.

There are limitations to this work. The use of pooled data means drug intake conditions, such as time of day, accompanying food and backbone regimen were standardised within but not necessarily across trials. Moreover, a number of studies involved healthy volunteers whilst others investigated HIV infected individuals,

although this was included as a covariate in the multivariate analyses. Whilst the majority of studies contributing data were Phase I clinical trials, one study was a Phase III trial (n=17) and only contributed DTG trough concentrations rather than intensive PK data, meaning the sample sizes for C_{max}/AUC_{0-24} and for C_{24} differed (N=76 and N=93 respectively). Additionally, two different assay methodologies were used for the PK analysis, potentially introducing variability. However, reassuringly, there was no significant difference in genotypic distribution for *ABCG2*, *UGT1A1* and *NR1I2* between groups analysed by either method. Findings need to be interpreted in the context of the limited population size and statistical power of this study. Finally, our population was predominantly Caucasian (75%) and whilst the genetic associations found were preserved when the analyses were restricted to Caucasians only, we could not conduct any other ethnicity sub-analyses due to the small numbers representing other ethnic groups, therefore clinical findings should be verified further in population-specific studies.

In conclusion, since there is evidence of concentration-dependent DTG side effects and given that DTG-based regimens are increasingly replacing preferred first line therapy in ARV naïve HIV patients worldwide, searching for genomic biomarkers of plasma exposure may help tailor DTG-based HIV therapy at individual and population levels. This study showed a pharmacogenetic association between DTG pharmacokinetics and variants in the *ABCG2*, *UGT1A1* and *NRI1/2* genes, particularly when combined. Further studies in large and diverse populations are warranted, particularly examining pharmacodynamic endpoints such as neuropsychiatric AEs, in order to further determine the clinical validity and population impact of pharmacogenetic testing for DTG.[371-373]

CHAPTER 7

General Discussion

Dolutegravir and cobicistat were approved for the treatment of HIV in 2013-14. Both addressed unmet clinical needs at the time and are now widely used. Their licences coincided with an intensification in the global ARV scale-up and, separately, a general paradigm shift in HIV research towards individualising therapy.[100, 101] Both drugs have played a significant role in these latest developments, particularly DTG, which replaced EFV as preferred first line agent in universal guidelines alongside the newly introduced recommendation to adopt a 'test and treat' approach.[64] It is estimated that at least 35 million people, including newly infected individuals, would need to be on treatment to meet in the 90–90–90 targets, meaning that, regardless of the target being reached and the residual need for further key population data (pregnancy, TB co-infection), the worldwide implementation of DTG is likely to be widespread, across many different settings and across very diverse populations.[65-68, 377] This highlights the importance of characterising the pharmacological behaviour of these drugs in real life settings and in commonly encountered clinical scenarios, in order to guide physicians in prescribing. It is particularly salient since data from licencing programs often only reflect drug use in highly selected groups of individuals under stringent trials conditions.[127]

The goal of HIV therapy is to achieve and maintain virological suppression. Whilst clinical developments in novel formulations such as long active (LA) injectables and implantables are currently at the forefront of research and results are promising, treatment today still requires lifelong daily oral ARV dosing.[378] Most agents now have near-perfect efficacy (>90%) in clinical trials in ARV naïve individuals, the chances of success of therapy therefore fall heavily on optimising adherence, itself dependent on a large number of factors (**figure 7.1**). The data presented in this thesis has

addressed gaps in knowledge within the domain of therapy-related factors that impact adherence, efficacy and tolerability of treatment, with the aim of assisting clinicians in appropriately individualising treatment to the patients and to their clinical circumstances.

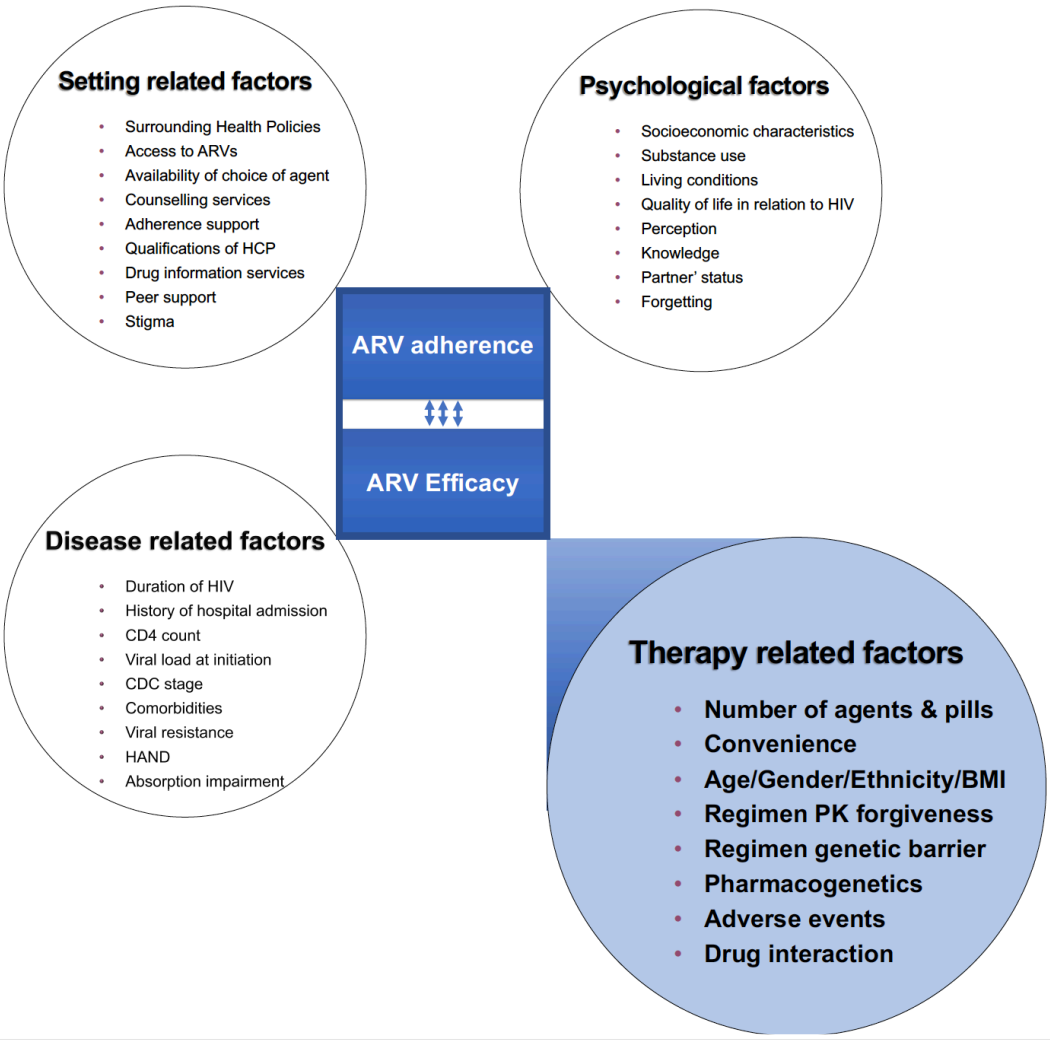


Figure 7.1: Non-exhaustive list of factors that influence adherence to and efficacy of ARVs. HCP: healthcare provider; CDC: Centre for Disease Control and Prevention; BMI: body mass index; PK: pharmacokinetics; ARV: antiretrovirals; HAND: HIV Associated Neurocognitive Disorder

As discussed in **chapter 2**, both high and low-to-middle income settings have seen an ageing of the HIV population as a result of greater survival and of increasing rates of infection in later life.[234-238] HIV physicians are now commonly faced with managing

ARVs in the context of multiple comorbidities and polypharmacy, both strongly associated with older age.[235-238, 379-385] Additionally, in advancing age, several physiological changes affect drug pharmacokinetics and pharmacodynamics.[239] Since DTG is a fitting candidate for therapy in this population, the study in **chapter 2** characterised DTG's intensive PK in PLWH ≥ 60 years, showing a significantly higher DTG C_{max} (25%) *vs* younger subjects (median age 36 years).

Changes in sleep and cognition were also evaluated over six months of DTG in this population. There were two discontinuations secondary to NP-AEs, matching published rates.[127] The one participant with PK data available had a C_{max}, AUC_{0-24} and C_{min} above the 95th centile for the study group, suggesting a PK contribution. This is in keeping with cohort data from the groups of Borghetti, Menard and Yagura, all of whom found an association between C_{min} and NP-AEs.[146, 148, 149, 232] However, there was no association between DTG PK parameters and changes in PD scores in the remaining subjects over time, in our study, which is in keeping with observations from Riva and Hoffman.[259, 151] More specifically, there were no changes in sleep scores in some subjects with very high drug concentrations (>95th centile) in whom, surprisingly, cognition improved significantly, suggesting that the mechanisms of DTG-related neurotoxicity are more complex than a simple linear or threshold-defined PK relationship. It may relate, as already discussed, to a combination of factors that include pharmacogenetic, immune and/or functional predispositions.[138] Further research is warranted in order to determine the role of DTG drug concentrations in its safety and tolerability profiles and to characterise other contributing factors more precisely. This will be crucial to minimising the personal and financial impacts of DTG adverse effects on individuals and health care systems, respectively.

As previously mentioned, there has been a recent drive to investigate treatment simplification strategies and challenge the use of conventional triple therapy, which has been the staple of HIV treatment since 1996. This aims to lessen toxicity, cost and, potentially, drug interactions through reducing the number of agents in any single regimen.[100] Treatment simplification is especially relevant in today's ageing HIV patient population.[236] One recent study conducted in France, demonstrated that DDIs are common in people over the age of 65 living with HIV and substantially increase healthcare cost by as much as $2693 per patient per year.[386] A number of simplification strategies have been examined, however, following the historically mixed success of PI/RTV monotherapy, concerns over CSF viral escape and poor quality data on DTG monotherapy, current data firmly favours dual therapy over monotherapy.[100-103, 387] Of note, phase 3 trials for long-acting dual therapy maintenance are on-going (cabotegravir + RPV).[388] RCT and cohort data available for some of the main dual combinations studied to date are discussed in **chapters 1 and 4**. The latter focuses on DTG combined with boosted DRV, which offers a simplified and safe regimen with a high genetic barrier and can particularly assist in cases where treatment simplification is desired but NRTI or NNRTI based dual therapy is not possible (e.g. cases of complex resistances).[101, 102] The **DUALIS** study and a number of cohort studies have already demonstrated high efficacy and safety of this combination as maintenance therapy, including in highly treatment experienced patients with resistance.[118-121] PK and efficacy data, however, were mainly available for DTG with DRV when boosted with RTV. **In chapter 4**, co-administration of DTG with DRV when boosted with COBI was therefore investigated; minimal intra-individual variance in DTG and DRV/COBI PK parameters when administered together was demonstrated (<10%)

compared to alone. Concentrations of both active agents remained manifold above the PA-IC_{90}/PA-EC_{90} in all participants, suggesting that no dose adjustment is required in either agents and that this combination can be prescribed safely, at standard doses, in the treatment of HIV-1, including in patients harbouring resistance. DTG/DRV/COBI thus offers a potent dual regimen that is taken as two tablets rather than three with RTV and is likely to become a strong addition to the HIV therapy armamentarium in future.

Despite advances to improve adherence such as single tablet regimens, smaller tablets, adherence support and purpose-designed technology, delayed or omitted doses still occur, potentially compromising virological control and risking the emergence of drug resistance. **In chapter 3**, the plasma PK of oral DTG and COBI-boosted EVG, DRV and ATV following cessation of drug intake were investigated, in order to understand and guide the management of late and missed doses for these drugs. A marked difference in the elimination rates of DTG and EVG was seen following treatment interruption. There was a significant decline in EVG GM concentration after 24 hours, paralleling a rapid COBI decay; EVG levels dropped to below the PA-IC_{95} shortly after the 36 hours mark. Conversely, DTG was persistent in systemic circulation for a very prolonged period, with GM concentrations remaining above the suggested plasma PA-IC_{90} for up to 72 hours and above the suggested MEC for over 48 hours post-drug intake cessation. These discrepancies in PK forgiveness may influence drug choices in patients who have suboptimal adherence, as clinical difference may emerge in this population. There was also an increase in rates of decline for ATV and DRV as COBI concentrations diminished. All 16 subjects in the study had ATV concentrations above or very close to the suggested MEC 30 hours post-dose, suggesting that a 6-hour drug

intake delay would not compromise optimal drug exposure and efficacy. DRV concentrations, however, appeared to drop below the MEC shortly after the 24-hour mark and 3/16 study individuals had concentrations below the suggested MEC cut-off 24 hours post-dose. DRV is a robust agent and whether this is clinically significant is unclear. PD data in patients who are poorly adherent to DRV/COBI are therefore needed. Overall, the findings contribute to the understanding of whether oral doses, for the specific drugs investigated, can be delayed or missed and, if so, to what extent. This is particularly important as DTG and the PIs are the agents used in patients who are susceptible to poor compliance or harbour viral resistance.[56, 260, 261]

PK tail data will be particularly important in the era of LA compounds, not only to determine optimal dosing intervals but also for clinicians to assess the suitability of certain patients for available options. There remains an ongoing debate on whether patients who struggle with adherence to medication and attendance to clinic would be suitable candidates for LA formulations of ARV agents with long PK tails. This is because of the risk of viral resistance in the context of prolonged exposure to subtherapeutic drug doses in those who do not attend their scheduled dosing. Consequently, strategies for the implementation of LA therapeutics in real life clinical settings is an important and actively evolving area.[388]

Chapter 5 describes the intra-individual variance in the PK of EE/LNG in one of the most commonly used oral contraceptive pill (Microgynon®) and of ATV/COBI co-formulated in Evotaz®, when they are administered together compared to alone. Evotaz® may be preferentially used over ATV + RTV in some patients in order to reduce pill burden.[208] With (TG)WLWH living longer and healthier lives, U=U

(undetectable = untransmittable) and the markedly minimised rates of mother-to-child transmission (MTCT) in the context of planned pregnancy with optimal viral suppression, access to safe and reliable contraception and ART is a priority on the HIV care agenda. As discussed comprehensively in **chapter 5**, oestrogen and progestogen have relatively narrow therapeutic indices and the tolerability, safety and efficacy sequalae of underexposure or overexposure to either are all potentially significant. This study demonstrated minimal changes in LNG concentrations and a smaller decrease in EE C_{min} than seen with ATV/RTV (25% *vs* 37%).[338, 339] There were no clinically significant changes in EE C_{max} or AUC_{0-24} (5% and 1% increases respectively). The disparity in DDI between the two pharmacological boosters is likely to relate to the fact that unlike RTV, COBI does not induce CYP2C9 and glucuronidation, both responsible for EE clearance.[198, 199, 338] ATV has fallen out of favour in high income countries but it remains in the WHO guidelines and large numbers of patients stable on ATV continue to take it. Whilst preliminary, these data are therefore important in informing physicians, who need to discuss and choose safe and reliable contraception with their female patients living with HIV. Whether this difference between ATV/RTV and ATV/COBI will be clinically significant, however, demands further characterisation.

Finally, considering that DTG is fast becoming the leading agent for naïve HIV patients in the transition to global ARV access, searching for genomic biomarkers of plasma exposure may help tailor DTG-based HIV therapy at individual and population level.[64, 65] **Chapter 6** therefore evaluated the impact of genetic variability in drug disposition genes on the PK of DTG, showing an independent pharmacogenetic association between DTG PK and variants in the *ABCG2*, *UGT1A1* and *NR1I2* genes,

particularly when combined. Most significantly, co-occurring *UGT1**28 and *NR1I2* c.63396C>T homozygosity was associated with a statistically significant 79% increase in $AUC_{0\text{-}24}$ and a 47% increase in C_{max} whilst combined *ABCG2* c.421C>A and *NR1I2* c.63396C>T variants were associated with a 43% increase in C_{max} and a 39% increase in $AUC_{0\text{-}24}$. The combination of *UGT1A1**28 poor metaboliser and *UGT1A1*6* intermediate metaboliser statuses correlated with a 43% increase in $AUC_{0\text{-}24}$. Overall, results indicate that genomic biomarkers of DTG plasma exposure may be best characterised with carefully defined sets of SNPs or scoring algorithms rather than with individual alleles. These findings warrant further research in large and diverse populations that particularly examine pharmacodynamic endpoints in order to determine the feasibility, clinical validity and population impact of pharmacogenetic testing for DTG.[228, 370-373]

There remains a call in the field for tangible biomarkers of DTG toxicity, particularly with regards to NP-AEs and to weight gain, both of which are likely to be multifactorial.[127, 144] From the studies of weight gain published so far, women and black people appear most likely to be at risk on InSTI-based regimen, particularly when compared with NNRTI-based regimen.[144] Median gain has ranged from 1kg to 4.9kg in RCTs that reported weight changes.[144] Recent data from IAS 2019, showed that DTG-related weight gain was significantly associated with TAF co-administration,[389] but, interestingly, there has also been a suggestion that DTG binding to the MC4R receptor may be involved. In vitro, DTG inhibited the binding of radiolabelled α-melanocyte-stimulating hormone (MSH) to the human recombinant MCR4 receptor by 64% at a concentration equal to the clinical $C_{max,}$ when >50% is considered clinically significant by the EMA. MC4R is involved in the regulation of

energy homeostasis and food intake, and deficiency in MC4R is associated with monogenic obesity.[390] It is not clear if these findings will be associated with changes in body weight in vivo and further studies are needed.[144] If a mechanistic link is found, MC4R genetic variants may merit further investigations to assess their contribution to DTG-mediated weight gain. For NP-AEs, the literature to date remains suggestive rather than conclusive. A number of cohort studies have reported that co-administration with ABC increases the risks of DTG discontinuation secondary to AEs, although this DDI has been refuted in a number of other studies, as have suggestions implicating PK, gender and age as risk factors.[129-135, 146] As previously discussed, Borghetti *et al.* reported an association between a common *SLC22A2* genetic variants and a set of measured subclinical NP-AEs during DTG therapy, although none led to drug termination.[232] In their study, two neuropsychiatric metrics were also associated with DTG C_{min}, leading to the authors proposing a synergy between DTG PK and genetic neurological susceptibility.[232] Yagura *et al.* similarly described an association between *UGT1A1**28 and *UGT1A1**6 gene polymorphisms and a higher cumulative incidence selected NP-AEs, mediated by C_{min} in a Japanese cohort.[148] These findings were furthered by data presented by the same authors in a poster at IAS 2019, showing an association between *UGT1A1**28 and *UGT1A1**6 gene polymorphisms and AE related DTG discontinuation one to four years after DTG initiation in a similar cohort.[149] There may be a genetic predisposition in the aetiology of DTG-mediated neurotoxicity. Further DTG NP-AE pharmacogenetic research is therefore warranted in order to investigate the role of SNPs, specifically in hard pharmacodynamic endpoints such as the discontinuation of DTG due to AEs with resolution of symptoms post-discontinuation. Genes involved in DTG transport across the BBB (e.g.BCRP) and in the susceptibility of neuronal receptors substrates

(e.g.OCT2) are of particular interest. This study is under-way and will characterise three SNPs in genes coding for DTG and BIC disposition (*ABCG2* (BCRP, rs2231142) and *UGT1A1**28) and coding for DTG target (*SLC22A2* (OCT2, rs316019)), in a large cohort of patients on DTG or BIC who have discontinued the drug secondary to NP-AEs (cases) compared to participants who have experienced no NP-AEs (controls) for over 1 year on either drug (the **DOLBIC** study).

Lastly, as summarised above, **chapter 3** reported a long DTG PK tail, with GM concentrations remaining above the *in-vitro* PA-IC_{90} for 3 days, above the suggested MEC for over 2 days and being detectable in plasma in 100% of participants at 4 days. Additionally, DTG C_{24} in all the DTG studies described in earlier chapters ranged between 1052 and 1324 ng/mL, for a 50mg OD dose, in various populations. This represents an inhibitory coefficient (IQ_{90}) ranging between 16 and 21, which is high for wild type virus. The IQ_{90} is used in evaluating a drug's efficacy at a given dose. It relates the *in-vivo* drug exposure to viral susceptibility, usually C_{min} divided by IC_{90} or IC_{95}, indicating the number of times the C_{min} is greater than the IC_{90}/IC_{95}. DTG GM C_{min} in the DTG SPC is 1110 ng/mL, giving an IQ_{90} within the same range as in our studies. For reference, the IQ_{95} for standard dose EFV is approximately 14 and NVP 12.[391] During the drug development phase for DTG, doses of 10 and 50 mg OD yielded C_{min} values that were 3 and 13 times higher than the PA-IC_{90}, in ARV naïve/ARV experienced, InSTI naïve patients, dosed with DTG monotherapy for 10 days.[390] The subsequent dose finding phase II study, SPRING1, evaluated 10mg, 25mg and 50mg OD against the active control efavirenz 600 mg in ARV-naive subjects. All regimens contained a NRTI backbone of ABC/3TC or TDF/FTC. At 48 weeks, all 3 DTG dosing arms achieved more rapid and sustained virological suppression than EFV (88-91% *vs*

82%) and there was no difference in efficacy between arms.[392] This raises the question of whether DTG could be considered for dose optimisation trials in first line therapy, particularly if PK is found to play a significant contributory role in DTG toxicity. Several dose optimisation trials have been conducted in the past to challenge approved supratherapeutic dosing of commonly used agents such at ATV, EFV and AZT.[393-396] In the ENCORE-1 study, for instance, lower dose of EFV, 400 mg OD, was non-inferior in efficacy and showed lower risks of EFV-related AEs compared to the approved dose of 600 mg OD, in ARV naïve individuals.[395, 396] Efficacy was consistent across different races and CYP2B6 polymorphisms, which are known to affect EFV concentrations. Efficacy results were subsequently replicated in patients with TB/HIV co-infection on rifampicin and in pregnant women and the WHO antiretroviral guidelines were updated to recommend EFV 400mg as the alternative first line option to DTG, aiming to reduce cost and overall toxicity of EFV-based therapy.[397-399] Consideration of DTG dose optimisation research aiming to reduce global cost and toxicity would depend on upcoming results from DTG toxicity aetiology studies, key population safety and efficacy data and overall cost-saving analyses, since DTG is already relatively competitively priced in LMIC (median price $30 per person per year; TDF/3TC/DTG is $75 per person-year).[66]

In conclusion, on the back of the remarkable advances made in HIV pharmacotherapy in the last three decades, the field has been able to progress towards investigating and implementing the individualisation of treatment. Tailoring of therapy can be beneficial on an individual level but also on a population-level, where characteristics such as genomics or clinical circumstances (e.g. co-infection and pregnancy) are common to a population. DTG and, to a lesser degree, COBI have played a significant role in this

paradigm shift. DTG, in particular, is becoming a key player in the global ARV scale up, meaning that any findings related to DTG will be applicable to very large populations. The novel data presented in this thesis addresses gaps in knowledge on DTG and COBI's pharmacological behaviour in important patient groups and clinical scenarios frequently encountered by physicians, namely older PLWH, women on contraception, patients who are poorly adherent, candidates for DTG/DRV/COBI dual therapy and genetically distinct populations. The findings provide data to assist clinicians in decision-making in clinically and genetically predefined sub-populations and support future research in DTG dose optimisation.

APPENDIX

- **Appendix 1: commercial nomenclature for individuals ARVs**
- **Appendix 2: supplementary material for chapter 2**

Appendix 1: commercial nomenclature for individuals ARVs

Molecule/combination	UK commercial name
Efavirenz + emtricitabine + tenofovir DF	Atripla
Bictegravir + TAF + emtricitabine	Biktarvy
Rilpivirine + emtricitabine + tenofovir DF	Eviplera
Rilpivirine + emtracitabine + TAF	Odefsey
Dolutegravir + abacavir + lamivudine	Triumeq
Elvitegravir + cobicistat+ emtracitabine + TAF	Genvoya
Darunavir + cobicistat + emtricitabine + TAF	Symtuza
Doravirine + lamivudine + tenofovir DF	Delstrigo
Dolutegravir + lamivudine	Dovato
Dolutegravir + rilpivirine	Juluca
Tenofovir DF + emtracitabine	Truvada
Tenofovir alafanemide (TAF)	Descovy
Abacavir + lamivudine	Kivexa
Efavirenz	Sustiva
Nevirapine	Viramune
Etravirine	Intelence
Rilpivirine	Edurant
Doravirine	Pifeltro
Raltegravir	Isentress
Dolutegravir	Tivicay
Maraviroc	Celsentri
Atazanavir	Reyataz
Darunavir	Prezista
Atazanavir + cobicistat	Evotaz
Darunavir + cobicistat	Rezolsta
Cobicistat	Tybost
Ritonavir	Norvir

Appendix 2: Description of sleep questionnaires used in chapter 2 study:

1. The Pittsburgh Sleep Quality Index (PSQI) is a well validated multidimensional tool with 19 scored questions (0-3 likert scale) covering 7 domains of subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications and daytime dysfunction. It aims to measure sleep quality and disturbances over the prior month and to discriminate between "good" and "poor" sleepers. The scoring range is 0-21. Score of 5 and above indicates poor sleep quality.
2. The insomnia severity scale (ISS) is a self-questionnaire to measure the nature, severity and impact of any insomnia experienced including associated concerns and distress; The scaling of items is on a 5 point likert scale, with a total range from 0-28; Cut offs are 0-7: no insomnia, 8-14: subthreshold insomnia, 15-21: significant insomnia and 22-28: severe insomnia.
3. The Epworth Sleepiness Scale (ESS) is an 8 question self reported tool to assess tendency for daytime sleepiness. It does not distinguish the cause and there is no recall period. The scoring range is between 0-24. Scores of 10 or higher indicate excessive daytime sleepiness.
4. The Functional Outcomes of Sleep Questionnaire (FOSQ) is a 30-question tool to assess the impact of excessive sleepiness on functional outcomes relevant to daily behaviours and sleep-related quality of life. It contains 30 items in 5 factor subscales: activity level, vigilance, intimacy and sexual relationships, general productivity, social outcome and difficulty performing a given activity. The range goes from 5-10. Higher scores indicate better functional status.
5. The Fatigue Severity Scale (FSS) is a 9-item questionnaire to assess the effect of fatigue on daytime function in a 7-point likert scale. There are no categories or

domains established and the recall period is 1 week. The scoring range goes from 0-63. Scores of 36 or higher indicate fatigue.

6. The SDQ is a 175-question extensive and comprehensive questionnaire, which assesses all of the sleep domains (this was only administered on days 1 and 180 only). The recall period is 6 months.

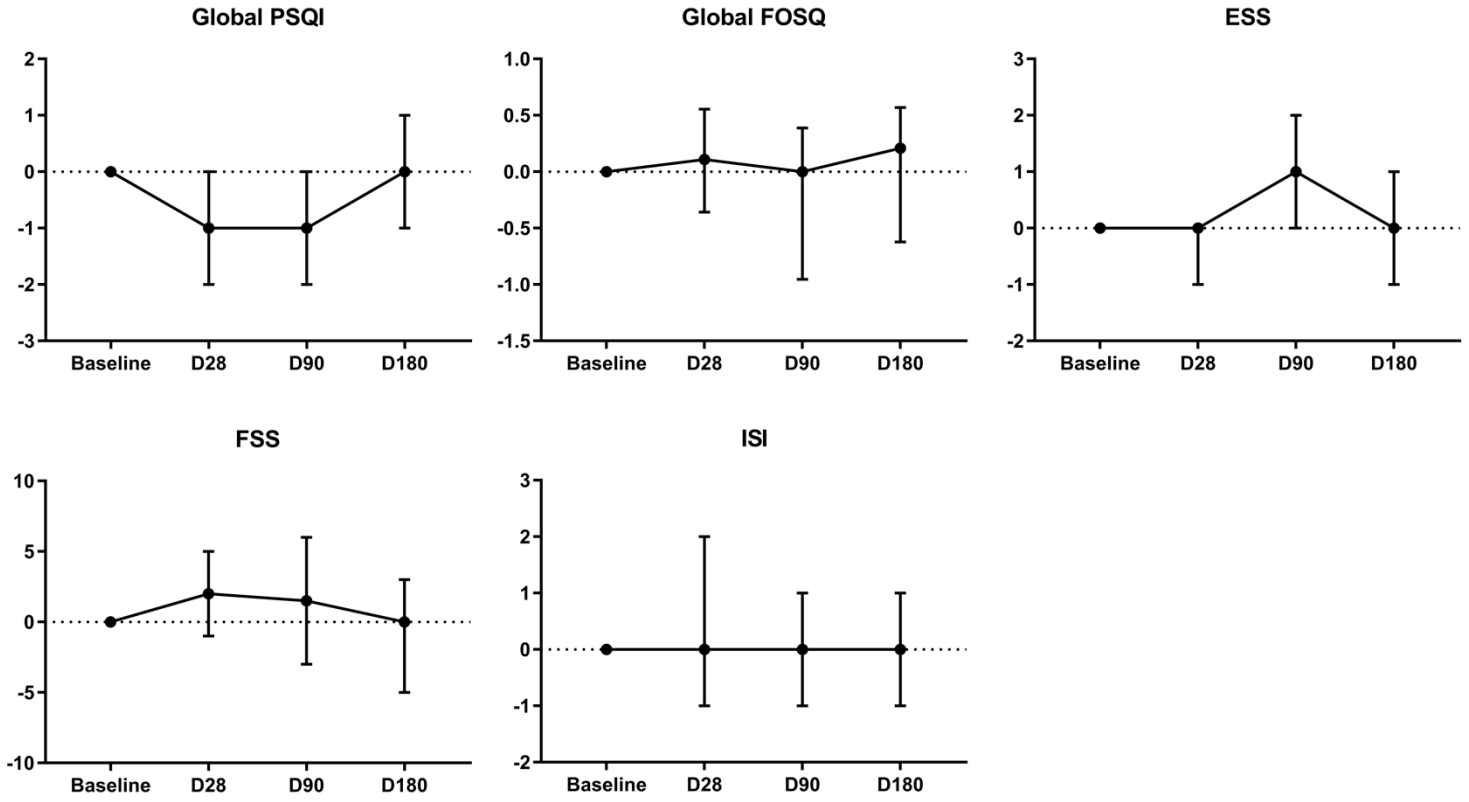
Appendix 2; Table A: Sleep questionnaire results by time point

Sleep component, median (IQR)	Baseline (n=43)		Day 28 (n=40)			Day 90 (n=38)			Day 180 (n=38)		
	n	Score	n	n'	Score	n	n'	Score	n	n'	Score
PSQI (Sleep Quality)											
Subjective sleep quality	43	1 (1,2)	40	40	1 (1,2)	37	37	1 (0,1)	38	38	1 (0,2)
Sleep latency	43	1 (0,1)	39	39	1 (0,2)	36	36	1 (0,1)	38	38	1 (0,2)
Sleep duration	42	1 (0,1)	39	38	1 (0,2)	37	37	1 (0,1)	36	35	1 (0,1.5)
Habitual sleep efficiency	40	0 (0,1.5)	38	35	1 (0,2)	37	35	0 (0,2)	35	32	1 (0,2)
Sleep disturbances	40	1 (1,2)	38	36	1 (1,2)	37	34	1 (1,2)	38	35	1 (1,2)
Sleeping medication	43	0 (0,0)	39	39	0 (0,0)	37	37	0 (0,0)	38	38	0 (0,0)
Daytime dysfunction	42	1 (0,1)	40	39	1 (0,1)	37	36	1 (0,1)	38	37	1 (0,1)
Global PSQI	36	5 (3.5,7.5)	35	29	6 (4,9)**	36	30	5.5 (3,9)	35	28	6 (4,11)
Global PSQI >5[a], n (%)	36	16 (44)	35	29	18 (51)	36	30	18 (50)	35	28	18 (51)
ESS (Sleepiness)											
Overall ESS	41	5 (3,10)	37	37	6 (4,8)	34	32	5 (2,7)	38	36	5 (3,8)
ESS ≥10[b], n (%)	41	12 (29)	37	37	8 (22)	34	32	6 (18)	38	36	9 (24)
ISI (Insomnia)											
Overall ISI	42	5.5 (2,10)	40	39	6.0 (3,10.5)	35	35	6.0 (1,10)	38	37	6.5 (1,12)
ISI category, n (%)											
≤14 (none or subthreshold)	42	38 (90)	40	39	37 (93)	35	35	33 (94)	38	37	30 (79)
15-21 (moderate)		3 (7)			2 (5)			2 (6)			8 (21)
≥22 (severe)		1 (2)			1 (3)			0 (0)			0 (0)
FOSQ (Functional Outcomes)											
General productivity	42	3.88 (3.6,4)	40	39	3.88 (3.6,4)	37	36	3.75 (3.25,4)	38	37	3.88 (3.6,4)
Social outcome	42	4.00 (4,4)	40	39	4.00 (4,4)	37	36	4.00 (4,4)	38	37	4.00 (4,4)
Activity level	43	3.67 (3.3,3.9)	40	40	3.56 (3.4,3.8)	37	37	3.67 (3.3,3.9)	38	38	3.65 (3.2,3.9)
Vigilence	43	3.71 (3,4)	40	40	3.63 (3.3,3.9)	37	37	3.71 (3.4,4)	38	38	3.57 (3.3,4)
Intimacy	38	3.75 (3,4)	36	35	3.75 (3.1,4)	34	31	4.00 (3,4)	36	31	3.88 (3,4)
Global FOSQ[d]	42	18.36 (16.1,19.5)	40	39	18.55 (16.7,19.4)	36	35	18.81 (17.8,19.7)	38	37	18.01 (16.4,19.6)
FSS (Fatigue)											
Overall FSS	39	22 (14,19)	35	31	23 (16,34)	31	28	21 (14,31)	35	31	26 (14,34)
FSS ≥36[c], n (%)	39	4 (10)	35	31	6 (17)	31	28	7 (23)	35	31	7 (20)

SDQ (Sleep Disorders)							
Sleep apnea	23	29 (25,31)			31	19	26 (23,31)
Periodic leg movement	38	20 (18,24)			36	32	20 (17,24)
Psychiatric sleep disorder	35	17 (14,21)			35	29	16 (14,21)
Narcolepsy	28	19 (17,22)			30	23	18 (16,20)

Scores are raw median (IQR) unless otherwise stated. N are number of individuals with results at time point. N' are number of individuals with baseline & timepoint results
Significant testing using Wilcoxon matched pairs sign-rank test compared to baseline. *** p <0.001, ** p <0.01, * p <0.05 (no correction for multiple testing)
EFV, efavirenz; ESS, Epworth sleepiness scale; FOSQ, Functional outcomes of sleep questionnaire; FSS, Fatigue severity scale of sleep disorders; ISI, Insomnia severity index; IQR, Interquartile range; PSQI, Pittsburgh sleep quality index; SDQ, Sleep disorders questionnaire
For all sleep components a higher score indicates poorer performance with the exception of FOSQ, for which a higher score indicates better performance. PSQI domain range 0-3, global range 0-21; FOSQ domain range 1-4, global range 5-20; ESS range 0-24; ISI range 0-28; FSS range 9-63; SDQ, sleep apnea range 12-60, period leg movement range 9-45, psychiatric sleep disorder range 9-45, narcolepsy range 15-75.
[a] Global PSQI >5 considered poor sleep quality; [b] ESS ≥10 considered sleepy; [c] FSS ≥36 indicates possible fatigue; [d] Global FOSQ only calculated if ≥60% questions answered

Appendix 2; Figure B: Changes in individual sleep questionnaire median scores from baseline to day 90 and 180 (95% CI)



Note: graphs show median (95% confidence interval) change from baseline. Sign reversed for all outcome measures where increasing values indicate performance decline so for all measures, negative values indicate performance decline & positive values indicate performance improvement

Appendix 2: Table C: Sleep scores by previous efavirenz status

Sleep component, median (IQR)	Baseline			Change from baseline, mean (SD)								
				Day 28			Day 90			Day 180		
	No EFV	EFV	*p-value*[a]	No EFV	EFV	*p-value*[b]	No EFV	EFV	*p-value*[b]	No EFV	EFV	*p-value*[b]
PSQI, global	6 (4,11.3)	4 (1,6)	*0.029**	-1 (-2,1)	-1 (-2.3,0.3)	*0.923*	-1 (-3,1)	-1 (-3,0)	*0.719*	0 (-1,1)	0 (-5,1)	*0.556*
FOSQ, global	17.3 (15.3,18.7)	19.4 (18.2,19.8)	*0.030**	0.11 (-0.8,1.0)	0.04 (-0.5,0.6)	*>0.999*	0.00 (-1.8,2.0)	-0.00 (-1.1,0.3)	*0.909*	0.52 (-1.0,2.2)	0.00 (-1.2,0.3)	*0.082*
ESS	6 (4.5,11.5)	4 (3,7.5)	*0.049**	0 (-2,2)	-1 (-1,0.5)	*0.674*	1 (-1,3)	1 (0,1)	*0.524*	-1 (-3,3)	0 (-1,1)	*0.664*
ISI	8.5 (3.8,11.5)	3 (0,7)	*0.015**	0 (-2,2.3)	0 (-3,2)	*0.961*	0.5 (-1.3,3.5)	0 (-4,1.5)	*0.225*	1 (-1,2.8)	-1 (-6,0)	*0.018**
FSS	24 (18,35)	21.5 (10,26)	*0.058*	2 (-4.3,5)	2 (-7,8)	*0.914*	1 (-5,8)	2 (-3.5,9)	*0.641*	1.5 (-6.5,5.5)	0 (-6,1)	*0.552*
SDQ, sleep apnea	31 (26,35)	26 (22,30)	*0.024**							2 (-2.5,4.5)	0 (-2.3,1)	*0.214*
SDQ, periodic leg movement	20 (18,24)	20.5 (17,21)	*0.953*							-0.5 (-1.3,3.3)	-0.5 (-4.3,2.3)	*0.350*
SDQ, psychiatric sleep disorder	17.5 (14,22)	16 (14,20)	*0.541*							1 (-3,3)	1 (-2.5,4.3)	*0.855*
SDQ, narcolepsy	18.5 (16,23.5)	20 (18,21)	*0.651*							-1.5 (-3,1.8)	3 (0,4)	*0.007***

Scores are raw median (IQR) unless otherwise stated.

EFV, efavirenz; ESS, Epworth sleepiness scale; FOSQ, Functional outcomes of sleep questionnaire; FSS, Fatigue severity scale of sleep disorders; ISI, Insomnia severity index; IQR, Interquartile range; PSQI, Pittsburgh sleep quality index; SDQ, Sleep disorders questionnaire

Note: At baseline, for all sleep components a higher score indicates poorer performance with the exception of FOSQ, for which a higher score indicates better performance. PSQI domain range 0-3, global range 0-21; FOSQ domain range 1-4, global range 5-20; ESS range 0-24; ISI range 0-28; FSS range 9-63; SDQ, sleep apnea range 12-60, period leg movement range 9-45, psychiatric sleep disorder range 9-45, narcolepsy range 15-75. For difference scores, score sign is reversed for all outcome measures where increasing values indicate performance decline. Thus, for all measures, negative change values indicate performace decline & positive values indicate performance improvement

[a]P-values are exact Mann-Whitney tests for difference between groups at baseline; [b]P-values are exact Mann-Whitney tests for absolute difference between groups (non-EFV versus EFV) for change from baseline to timepoint (no correction for multiple testing)

Appendix 2; Table E: P-values for correlation between PK parameters and change in sleep scores (baseline-day 180)

Sleep component	C_{max}	C_{last}	AUC_{last}
PSQI, global	-0.16	-0.1	0.15
FOSQ, global	0.04	0.07	0.09
ESS	-0.1	-0.21	-0.12
ISI	-0.12	-0.15	-0.14
FSS	-0.03	0.06	-0.02

Correlations tested using Spearman correlation. *** p <0.001, ** p <0.01 * p <0.0

Appendix 2; Table D: P-values for correlation between PK parameters at day 28 and sleep scores at day 180

Sleep component	C_{max}	C_{last}	AUC_{last}
PSQI, global	-0.04	-0.09	-0.12
FOSQ, global	-0.09	0.24	0.17
ESS	0.17	-0.15	-0.02
ISI	0.01	0.08	0.01
FSS	-0.06	0.17	0.45

Correlations tested using Spearman correlation. *** p <0.001, ** p <0.01 * p <0.0

REFERENCES

1. UNAIDS. Fact sheet - Latest statistics on the status of the AIDS epidemic. Accessed 15 June 2018. Available at: http://www.unaids.org/en/resources/fact-sheet.
2. Flexner C. Antiretroviral Agents and Treatment of HIV Infection. In: Bruton L (ed) *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 13th ed. New York: McGraw Hill, 2018, pp.1623.
3. Hoen E, Berger J, Calmy A, et al. Driving a decade of change: HIV/AIDS, patents and access to medicines for all. *J Int AIDS Soc* 2011; 14: 15. DOI: 10.1186/1758-2652-14-15.
4. Rodger AJ, Cambiano V, Bruun T, et al. Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy. *JAMA* 2016; 316: 171-181. DOI: 10.1001/jama.2016.5148.
5. Chasela CS, Hudgens MG, Jamieson DJ, et al. Maternal or infant antiretroviral drugs to reduce HIV-1 transmission. *N Engl J Med* 2010; 362: 2271-2281. DOI: 10.1056/NEJMoa0911486.
6. Geretti AM, Rodger AJ and Lundgren J. HIV Transmission During Condomless Sex With a Seropositive Partner With Suppressed Infection-Reply. *JAMA* 2016; 316: 2045. DOI: 10.1001/jama.2016.16033.
7. World Health Organisation. Global health sector strategy on HIV, 2016-2021. Accessed 15th June 2018. Available at: http://www.who.int/hiv/strategy2016-2021/ghss-hiv/en/.
8. Hymes KB, Cheung T, Greene JB, et al. Kaposi's sarcoma in homosexual men-a report of eight cases. *Lancet* 1981; 2: 598-600.
9. Centers for Disease Control (CDC) (1982, 9 July) 'Opportunistic infections and Kaposi's Sarcoma among Haitians in the United States' 31(26):353-4,360-1.
10. Centers for Disease Control. Pneumocystis pneumonia - Los Angeles. MMWR Morb Mortal Wkly Rep 1981; 30(21): 250-2.
11. Greene WC. A history of AIDS: looking back to see ahead. *Eur J Immunol* 2007; 37 Suppl 1: S94-102. DOI: 10.1002/eji.200737441.
12. Nahmias AJ, Weiss J, Yao X, et al. Evidence for human infection with an HTLV III/LAV-like virus in Central Africa, 1959. *Lancet* 1986; 1: 1279-1280.
13. Oldest Surviving HIV Virus Tells All, By Science News Staff. Feb. 3, 1998. Accessed 15 June 2018. Available at: http://www.sciencemag.org/news/1998/02/oldest-surviving-hiv-virus-tells-all.
14. Pepin J. The origins of AIDS. Cambridge, UK ; New York: Cambridge University Press; 2011. xiv, 293 p. p.
15. Faria NR, Rambaut A, Suchard MA, et al. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. *Science* 2014; 346: 56-61. DOI: 10.1126/science.1256739.
16. Gao F, Bailes E, Robertson DL, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. *Nature* 1999; 397: 436-441. DOI: 10.1038/17130.
17. Sharp PM and Hahn BH. Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med* 2011; 1: a006841. DOI: 10.1101/cshperspect.a006841.
18. Drucker E, Alcabes PG and Marx PA. The injection century: massive unsterile injections and the emergence of human pathogens. *Lancet* 2001; 358: 1989-1992. DOI: 10.1016/S0140-6736(01)06967-7.

19. Hahn BH, Shaw GM, De Cock KM, et al. AIDS as a zoonosis: scientific and public health implications. *Science* 2000; 287: 607-614.
20. Marx PA, Alcabes PG and Drucker E. Serial human passage of simian immunodeficiency virus by unsterile injections and the emergence of epidemic human immunodeficiency virus in Africa. *Philos Trans R Soc Lond B Biol Sci* 2001; 356: 911-920. DOI: 10.1098/rstb.2001.0867.
21. Fischl MA, Richman DD, Grieco MH, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med* 1987; 317: 185-191. DOI: 10.1056/NEJM198707233170401.
22. Wright K. AIDS therapy. First tentative signs of therapeutic promise. *Nature* 1986; 323: 283. DOI: 10.1038/323283a0.
23. Wright K. AIDS protein made. *Nature* 1986; 319: 525. DOI: 10.1038/319525b0.
24. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983; 220: 868-871.
25. Gallo RC, Sarin PS, Gelmann EP, et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science* 1983; 220: 865-867.
26. Coffin JM, Hughes SH and Varmus H. *Retroviruses*. Plainview, N.Y.: Cold Spring Harbor Laboratory Press, 1997, p.xv, 843 p.
27. Aldrich C and Hemelaar J. Global HIV-1 diversity surveillance. *Trends Mol Med* 2012; 18: 691-694. DOI: 10.1016/j.molmed.2012.06.004.
28. Lau KA and Wong JJ. Current Trends of HIV Recombination Worldwide. *Infect Dis Rep* 2013; 5: e4. DOI: 10.4081/idr.2013.s1.e4.
29. Sierra S, Kupfer B and Kaiser R. Basics of the virology of HIV-1 and its replication. *J Clin Virol* 2005; 34: 233-244. DOI: 10.1016/j.jcv.2005.09.004.
30. Goto T, Nakai M and Ikuta K. The life-cycle of human immunodeficiency virus type 1. *Micron* 1998; 29: 123-138.
31. Li G, Piampongsant S, Faria NR, et al. An integrated map of HIV genome-wide variation from a population perspective. *Retrovirology* 2015; 12: 18. DOI: 10.1186/s12977-015-0148-6.
32. Arts EJ and Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb Perspect Med* 2012; 2: a007161. DOI: 10.1101/cshperspect.a007161.
33. Castro-Gonzalez S, Colomer-Lluch M and Serra-Moreno R. Barriers for HIV Cure: The Latent Reservoir. *AIDS Res Hum Retroviruses* 2018; 34: 739-759. 2018/07/31. DOI: 10.1089/AID.2018.0118.
34. Shafer RW. Human Immunodeficiency Virus Type 1 Drug Resistance Mutations Update. *J Infect Dis* 2017; 216: S843-S846. DOI: 10.1093/infdis/jix398.
35. Grandgenett DP, Pandey KK, Bera S, et al. Multifunctional facets of retrovirus integrase. *World J Biol Chem* 2015; 6: 83-94. DOI: 10.4331/wjbc.v6.i3.83.
36. Engelman A and Cherepanov P. Retroviral Integrase Structure and DNA Recombination Mechanism. *Microbiol Spectr* 2014; 2. DOI: 10.1128/microbiolspec.MDNA3-0024-2014.
37. Ciuffi A. The benefits of integration. *Clin Microbiol Infect* 2016; 22: 324-332. DOI: 10.1016/j.cmi.2016.02.013.
38. Hare S, Gupta SS, Valkov E, et al. Retroviral intasome assembly and inhibition of DNA strand transfer. *Nature* 2010; 464: 232-236. DOI: 10.1038/nature08784.

39. Maertens GN, Hare S and Cherepanov P. The mechanism of retroviral integration from X-ray structures of its key intermediates. *Nature* 2010; 468: 326-329. DOI: 10.1038/nature09517.
40. Cai M, Zheng R, Caffrey M, et al. Solution structure of the N-terminal zinc binding domain of HIV-1 integrase. *Nat Struct Biol* 1997; 4: 567-577.
41. Eijkelenboom AP, van den Ent FM, Vos A, et al. The solution structure of the amino-terminal HHCC domain of HIV-2 integrase: a three-helix bundle stabilized by zinc. *Curr Biol* 1997; 7: 739-746.
42. Dyda F, Hickman AB, Jenkins TM, et al. Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. *Science* 1994; 266: 1981-1986.
43. Eijkelenboom AP, Lutzke RA, Boelens R, et al. The DNA-binding domain of HIV-1 integrase has an SH3-like fold. *Nat Struct Biol* 1995; 2: 807-810.
44. Lodi PJ, Ernst JA, Kuszewski J, et al. Solution structure of the DNA binding domain of HIV-1 integrase. *Biochemistry* 1995; 34: 9826-9833.
45. Puras Lutzke RA, Eppens NA, Weber PA, et al. Identification of a hexapeptide inhibitor of the human immunodeficiency virus integrase protein by using a combinatorial chemical library. *Proc Natl Acad Sci U S A* 1995; 92: 11456-11460.
46. Ojwang JO, Buckheit RW, Pommier Y, et al. T30177, an oligonucleotide stabilized by an intramolecular guanosine octet, is a potent inhibitor of laboratory strains and clinical isolates of human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 1995; 39: 2426-2435.
47. Hazuda DJ, Felock P, Witmer M, et al. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* 2000; 287: 646-650.
48. Espeseth AS, Felock P, Wolfe A, et al. HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. *Proc Natl Acad Sci U S A* 2000; 97: 11244-11249. DOI: 10.1073/pnas.200139397.
49. Lataillade M and Kozal MJ. The hunt for HIV-1 integrase inhibitors. *AIDS Patient Care STDS* 2006; 20: 489-501. DOI: 10.1089/apc.2006.20.489.
50. Tivicay (Dolutegravir 50mg). ViiV Healthcare Ltd. Stockley Park West, Uxbridge, Middlesex, UB11 1BT, UK. Accessed on 7th June 2018. Available at: https://www.medicines.org.uk/emc/product/5248.
51. Biktarvy 50 mg/200 mg/25 mg film-coated tablets (tenofovir alafenamide fumarate/emtricitabine/bictegravir). Gilead Sciences Ltd. Gilead Medical Information (UK & Eire), 280 High Holborn, London, WC1V 7EE, UK. Available at: https://www.medicines.org.uk/emc/product/9313. Access on: 24 July 2018.
52. Messiaen P, Wensing AM, Fun A, et al. Clinical use of HIV integrase inhibitors: a systematic review and meta-analysis. *PLoS One* 2013; 8: e52562. DOI: 10.1371/journal.pone.0052562.
53. Günthard HF SM, Benson CA, et al. Antiretroviral drugs for treatment and prevention of HIV Infection in adults: 2016 recommendations of the International Antiviral Society-USA Panel. . *JAMA* 2016; 316:191-210.
54. U.S. Department of Health and Human Services. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Aids *Info.* Last Updated: May 30, 2018. Available at: https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv/0. Accesses on 23 June 2018. .

55. Battegay M LJ, Ryom L. European AIDS Clinical Society. Clinical management and treatment of HIV-infected patients in Europe. V 9.0. October 2017. Available from: http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html. Accessed 4 July 2018.
56. Waters L. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015 (2016 interim update). http://www.bhiva.org/documents/Guidelines/Treatment/2016/treatment-guidelines-2016-interim-update.pdf. BHIVA. Accessed 19 December 2017.
57. Podany AT, Scarsi KK and Fletcher CV. Comparative Clinical Pharmacokinetics and Pharmacodynamics of HIV-1 Integrase Strand Transfer Inhibitors. *Clin Pharmacokinet* 2017; 56: 25-40. DOI: 10.1007/s40262-016-0424-1.
58. Elliot E, Chirwa M and Boffito M. How recent findings on the pharmacokinetics and pharmacodynamics of integrase inhibitors can inform clinical use. *Curr Opin Infect Dis* 2017; 30: 58-73. DOI: 10.1097/QCO.0000000000000327.
59. Min S, Sloan L, DeJesus E, et al. Antiviral activity, safety, and pharmacokinetics/pharmacodynamics of dolutegravir as 10-day monotherapy in HIV-1-infected adults. *AIDS* 2011; 25: 1737-1745. DOI: 10.1097/QAD.0b013e32834a1dd9.
60. Min S, Song I, Borland J, et al. Pharmacokinetics and safety of S/GSK1349572, a next-generation HIV integrase inhibitor, in healthy volunteers. *Antimicrob Agents Chemother* 2010; 54: 254-258. DOI: 10.1128/AAC.00842-09.
61. Taha H, Das A and Das S. Clinical effectiveness of dolutegravir in the treatment of HIV/AIDS. *Infect Drug Resist* 2015; 8: 339-352. DOI: 10.2147/IDR.S68396.
62. Cruciani M and Parisi SG. Dolutegravir based antiretroviral therapy compared to other combined antiretroviral regimens for the treatment of HIV-infected naive patients: A systematic review and meta-analysis. *PLoS One* 2019; 14: e0222229. 2019/09/11. DOI: 10.1371/journal.pone.0222229.
63. World Health Organization. Transition to new antiretrovirals in HIV programmes; 2017. Available at: http://apps.who.int/iris/bitstream/10665/255888/1/WHOHIV-2017.20-eng.pdf?ua=1 (accessed March 2018).
64. World Health Organization. Updated Recommendations On First-Line And Second-Line Antiretroviral Regimens And Post-Exposure Prophylaxis And Recommendations On Early Infant Diagnosis Of HIV. Published July 2018. Accessed August 2018. Available at: http://apps.who.int/iris/bitstream/handle/10665/273632/WHO-CDS-HIV-18.18-eng.pdf?ua=1.
65. Hill A, Clayden P, Thorne C, et al. Safety and pharmacokinetics of dolutegravir in HIV-positive pregnant women: a systematic review. *J Virus Erad* 2018; 4: 66-71.
66. Vitoria M, Hill A, Ford N, et al. The transition to dolutegravir and other new antiretrovirals in low-income and middle-income countries: what are the issues? *AIDS* 2018; 32: 1551-1561. 2018/05/11. DOI: 10.1097/QAD.0000000000001845.
67. UNITAID. New high-quality antiretroviral therapy to be launched in South Africa, Kenya and over 90 low- and middle-income countries at reduced price; 2017. Available at: https://unitaid.eu/news-blog/new-high-quality antiretroviraltherapy-launched-south-africa-kenya-90-low-middle-income-countries-reducedprice/# en. Accessed August 2018.

68. Clinton Health Access Initiative. ARV market report; 2017. Available at: https://clintonhealthaccess.org/content/uploads/2017/09/2017-ARV-Market-Report_Final-2.pdf. Accessed Auguest 2018.
69. Walmsley SL, Antela A, Clumeck N, et al. Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection. *N Engl J Med* 2013; 369: 1807-1818. DOI: 10.1056/NEJMoa1215541.
70. Raffi F, Jaeger H, Quiros-Roldan E, et al. Once-daily dolutegravir versus twice-daily raltegravir in antiretroviral-naive adults with HIV-1 infection (SPRING-2 study): 96 week results from a randomised, double-blind, non-inferiority trial. *Lancet Infect Dis* 2013; 13: 927-935. DOI: 10.1016/S1473-3099(13)70257-3.
71. Cahn P, Pozniak AL, Mingrone H, et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. *Lancet* 2013; 382: 700-708. DOI: 10.1016/S0140-6736(13)61221-0.
72. Castagna A, Maggiolo F, Penco G, et al. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. *J Infect Dis* 2014; 210: 354-362. DOI: 10.1093/infdis/jiu051.
73. Molina JM, Clotet B, van Lunzen J, et al. Once-daily dolutegravir versus darunavir plus ritonavir for treatment-naive adults with HIV-1 infection (FLAMINGO): 96 week results from a randomised, open-label, phase 3b study. *Lancet HIV* 2015; 2: e127-136. DOI: 10.1016/S2352-3018(15)00027-2.
74. Wong E, Trustman N and Yalong A. HIV pharmacotherapy: A review of integrase inhibitors. *JAAPA* 2016; 29: 36-40. DOI: 10.1097/01.JAA.0000475465.07971.19.
75. Triumeq 50mg, 300mg, 600mg Tablets (lamivudine, abacavir, dolutegravir). ViiV healthcare Ltd, Stockley Park West, Uxbridge, Middlesex,, UB11 1BT, UK. Available at: https://www.medicines.org.uk/emc/product/3318/smpc. Accessed: 4 August 2018.
76. Cottrell ML, Hadzic T and Kashuba AD. Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir. *Clin Pharmacokinet* 2013; 52: 981-994. DOI: 10.1007/s40262-013-0093-2.
77. Castellino S, Moss L, Wagner D, et al. Metabolism, excretion, and mass balance of the HIV-1 integrase inhibitor dolutegravir in humans. *Antimicrob Agents Chemother* 2013; 57: 3536-3546. DOI: 10.1128/AAC.00292-13.
78. Adams JL, Patterson KB, Prince HM, et al. Single and multiple dose pharmacokinetics of dolutegravir in the genital tract of HIV-negative women. *Antivir Ther* 2013; 18: 1005-1013. DOI: 10.3851/IMP2665.
79. Greener BN, Patterson KB, Prince HM, et al. Dolutegravir pharmacokinetics in the genital tract and colorectum of HIV-negative men after single and multiple dosing. *J Acquir Immune Defic Syndr* 2013; 64: 39-44. DOI: 10.1097/QAI.0b013e31829ed7a4.
80. Letendre SL, Mills AM, Tashima KT, et al. ING116070: a study of the pharmacokinetics and antiviral activity of dolutegravir in cerebrospinal fluid in HIV-1-infected, antiretroviral therapy-naive subjects. *Clin Infect Dis* 2014; 59: 1032-1037. DOI: 10.1093/cid/ciu477.
81. Song I, Borland J, Chen S, et al. Effect of food on the pharmacokinetics of the integrase inhibitor dolutegravir. *Antimicrob Agents Chemother* 2012; 56: 1627-1629. DOI: 10.1128/AAC.05739-11.

82. Patel P, Song I, Borland J, et al. Pharmacokinetics of the HIV integrase inhibitor S/GSK1349572 co-administered with acid-reducing agents and multivitamins in healthy volunteers. *J Antimicrob Chemother* 2011; 66: 1567-1572. DOI: 10.1093/jac/dkr139.
83. Hightower KE, Wang R, Deanda F, et al. Dolutegravir (S/GSK1349572) exhibits significantly slower dissociation than raltegravir and elvitegravir from wild-type and integrase inhibitor-resistant HIV-1 integrase-DNA complexes. *Antimicrob Agents Chemother* 2011; 55: 4552-4559. DOI: 10.1128/AAC.00157-11.
84. Hare S, Smith SJ, Metifiot M, et al. Structural and functional analyses of the second-generation integrase strand transfer inhibitor dolutegravir (S/GSK1349572). *Mol Pharmacol* 2011; 80: 565-572. DOI: 10.1124/mol.111.073189.
85. Quashie PK, Mesplede T, Han YS, et al. Biochemical analysis of the role of G118R-linked dolutegravir drug resistance substitutions in HIV-1 integrase. *Antimicrob Agents Chemother* 2013; 57: 6223-6235. DOI: 10.1128/AAC.01835-13.
86. Wainberg MA, Han YS and Mesplede T. Might dolutegravir be part of a functional cure for HIV? *Can J Microbiol* 2016; 62: 375-382. DOI: 10.1139/cjm-2015-0725.
87. Marcelin AG, Grude M, Charpentier C, et al. Resistance to integrase inhibitors: a national study in HIV-1-infected treatment-naive and -experienced patients. *J Antimicrob Chemother* 2019; 74: 1368-1375. 2019/02/23. DOI: 10.1093/jac/dkz021.
88. Mesplede T and Wainberg MA. Resistance against Integrase Strand Transfer Inhibitors and Relevance to HIV Persistence. *Viruses* 2015; 7: 3703-3718. DOI: 10.3390/v7072790.
89. Wainberg MA and Han YS. Will drug resistance against dolutegravir in initial therapy ever occur? *Front Pharmacol* 2015; 6: 90. DOI: 10.3389/fphar.2015.00090.
90. Lubke N, Jensen B, Huttig F, et al. Failure of Dolutegravir First-Line ART with Selection of Virus Carrying R263K and G118R. *N Engl J Med* 2019; 381: 887-889. 2019/08/29. DOI: 10.1056/NEJMc1806554.
91. Kobayashi M, Yoshinaga T, Seki T, et al. In Vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. *Antimicrob Agents Chemother* 2011; 55: 813-821. DOI: 10.1128/AAC.01209-10.
92. Quashie PK, Mesplede T and Wainberg MA. Evolution of HIV integrase resistance mutations. *Curr Opin Infect Dis* 2013; 26: 43-49. DOI: 10.1097/QCO.0b013e32835ba81c.
93. Quashie PK, Mesplede T, Han YS, et al. Characterization of the R263K mutation in HIV-1 integrase that confers low-level resistance to the second-generation integrase strand transfer inhibitor dolutegravir. *J Virol* 2012; 86: 2696-2705. DOI: 10.1128/JVI.06591-11.
94. Anstett K, Fusco R, Cutillas V, et al. Dolutegravir-Selected HIV-1 Containing the N155H and R263K Resistance Substitutions Does Not Acquire Additional Compensatory Mutations under Drug Pressure That Lead to Higher-Level Resistance and Increased Replicative Capacity. *J Virol* 2015; 89: 10482-10488. DOI: 10.1128/JVI.01725-15.
95. Carganico A, Dupke S, Ehret R, et al. New dolutegravir resistance pattern identified in a patient failing antiretroviral therapy. *J Int AIDS Soc* 2014; 17: 19749. DOI: 10.7448/IAS.17.4.19749.

96. Cavalcanti Jde S, Ferreira JL, Guimaraes PM, et al. High frequency of dolutegravir resistance in patients failing a raltegravir-containing salvage regimen. *J Antimicrob Chemother* 2015; 70: 926-929. DOI: 10.1093/jac/dku439.
97. Liang J, Mesplede T, Oliveira M, et al. The Combination of the R263K and T66I Resistance Substitutions in HIV-1 Integrase Is Incompatible with High-Level Viral Replication and the Development of High-Level Drug Resistance. *J Virol* 2015; 89: 11269-11274. DOI: 10.1128/JVI.01881-15.
98. Mesplede T, Quashie PK, Osman N, et al. Viral fitness cost prevents HIV-1 from evading dolutegravir drug pressure. *Retrovirology* 2013; 10: 22. DOI: 10.1186/1742-4690-10-22.
99. Wares M, Mesplede T, Quashie PK, et al. The M50I polymorphic substitution in association with the R263K mutation in HIV-1 subtype B integrase increases drug resistance but does not restore viral replicative fitness. *Retrovirology* 2014; 11: 7. DOI: 10.1186/1742-4690-11-7.
100. Baril JG, Angel JB, Gill MJ, et al. Dual Therapy Treatment Strategies for the Management of Patients Infected with HIV: A Systematic Review of Current Evidence in ARV-Naive or ARV-Experienced, Virologically Suppressed Patients. *PLoS One* 2016; 11: e0148231. DOI: 10.1371/journal.pone.0148231.
101. Katlama C, Ghosn J and Murphy RL. Individualized antiretroviral therapeutic approaches: less can be more. *AIDS* 2017; 31: 1065-1071. 2017/03/31. DOI: 10.1097/QAD.0000000000001476.
102. Pasquau J and Hidalgo-Tenorio C. Nuke-Sparing Regimens for the Long-Term Care of HIV Infection. *AIDS Rev* 2015; 17: 220-230.
103. de Miguel Buckley R, Montejano R, Stella-Ascariz N, et al. New Strategies of ARV: the Road to Simplification. *Curr HIV/AIDS Rep* 2018; 15: 11-19. DOI: 10.1007/s11904-018-0371-6.
104. Cento V and Perno CF. Two-drug regimens with dolutegravir plus rilpivirine or lamivudine in HIV-1 treatment-naive, virologically-suppressed patients: latest evidences from the literature on their efficacy and safety. *J Glob Antimicrob Resist* 2019 2019/08/26. DOI: 10.1016/j.jgar.2019.08.010.
105. Juluca, Dolutegravir/Rilpivirine 50 mg/25 mg. ViiV Healthcare, Stockley Park West, Uxbridge, Middlesex,, UB11 1BT, UK. Available at https://www.medicines.org.uk/emc/product/9246 (Consulted 30th June 2019).
106. Cahn P, Rolon MJ, Figueroa MI, et al. Dolutegravir-lamivudine as initial therapy in HIV-1 infected, ARV-naive patients, 48-week results of the PADDLE (Pilot Antiretroviral Design with Dolutegravir LamivudinE) study. *J Int AIDS Soc* 2017; 20: 21678. DOI: 10.7448/IAS.20.01.21678.
107. Taiwo BO, Marconi VC, Berzins B, et al. Dolutegravir plus lamivudine maintain HIV-1 suppression through week 48 in a pilot randomized trial. *Clin Infect Dis* 2017. DOI: 10.1093/cid/cix1131.
108. Cahn P, Sierra Madero J, Arribas J, et al. Non-inferior efficacy of dolutegravir (DTG) plus lamivudine (3TC) versus DTG plus tenofovir/emtricitabine (TDF/FTC) fixed-dose combination in antiretroviral treatment-naïve adults with HIV-1 infection - 48-week results from the GEMINI studies. Program and abstracts of the 22nd International AIDS Conference; July 23-27, 2018; Amsterdam, the Netherlands. Abstract TUAB0106LB.
109. Joly V BC, Landman R, Raffi F, Katlama C, Cabié A, et al. Promising results of dolutegravir + lamivudine maintenance in ANRS 167 LAMIDOL trial. Conference on Retroviruses and Opportunistic Infections; February 13–16, 2017; Seattle. Abstract 458.

110. Taiwo BO, Zheng L, Nyaku AN, Stefanescu A, Sax PE, Haas D. ACTG A5353: a pilot study of dolutegravir (DTG) + lamivudine (3TC) for initial treatment of HIV-1-infected participants with HIV-1 RNA < 500,000 copies/mL. *Abstracts from 9th International AIDS Sociey Conference on HIV Science (IAS 2017). Paris July 2017.* Abstract MOAB0107LB.
111. Ford SL, Gould E, Chen S, et al. Lack of pharmacokinetic interaction between rilpivirine and integrase inhibitors dolutegravir and GSK1265744. *Antimicrob Agents Chemother* 2013; 57: 5472-5477. DOI: 10.1128/AAC.01235-13.
112. Llibre JM, Hung CC, Brinson C, et al. Efficacy, safety, and tolerability of dolutegravir-rilpivirine for the maintenance of virological suppression in adults with HIV-1: phase 3, randomised, non-inferiority SWORD-1 and SWORD-2 studies. *Lancet* 2018; 391: 839-849. DOI: 10.1016/S0140-6736(17)33095-7.
113. Casado JL, Monsalvo M, Rojo AM, et al. Dolutegravir and rilpivirine for the maintenance treatment of virologically suppressed HIV-1 infection. *Expert Rev Clin Pharmacol* 2018; 11: 561-570. DOI: 10.1080/17512433.2018.1478726.
114. Capetti AF, Cossu MV, Sterrantino G, et al. Dolutegravir Plus Rilpivirine as a Switch Option in cART-Experienced Patients: 96-Week Data. *Ann Pharmacother* 2018; 52: 740-746. 2018/02/28. DOI: 10.1177/1060028018761600.
115. Gantner P, Cuzin L, Allavena C, et al. Efficacy and safety of dolutegravir and rilpivirine dual therapy as a simplification strategy: a cohort study. *HIV Med* 2017; 18: 704-708. DOI: 10.1111/hiv.12506.
116. Capetti AF, Cossu MV, Paladini L, et al. Dolutegravir plus rilpivirine dual therapy in treating HIV-1 infection. *Expert Opin Pharmacother* 2018; 19: 65-77. DOI: 10.1080/14656566.2017.1417984.
117. Díaz AC, J.L.; Dronda, F.; Gómez-Ayerbe, C. et al. Dolutegravir plus rilpivirine in suppressed heavily pre-treated HIV-infected patients. 21st International AIDS Conference. Durban, South Africa, abstract TUPDB0106, 2016.
118. Spinner CKm, T.; Schneider, J. *et al*. A switch to dolutegravir in combination with boosted darunavir is safe and effective in suppressed patients with HIV - a subanalysis of the dualis study. 10th IAS Conference on HIV Science, Mexico City, abstract MOPEB246, 2019.
119. Wheeler J, Chan S, Harrigan PR, et al. Dolutegravir with boosted darunavir treatment simplification for the transmitted HIV thymidine analog resistance in Manitoba, Canada. *Int J STD AIDS* 2018; 29: 520-522. DOI: 10.1177/0956462418760426.
120. Capetti AF, Cossu MV, Orofino G, et al. A dual regimen of ritonavir/darunavir plus dolutegravir for rescue or simplification of rescue therapy: 48 weeks' observational data. *BMC Infect Dis* 2017; 17: 658. DOI: 10.1186/s12879-017-2755-4.
121. Hawkins KL, Montague BT, Rowan SE, et al. Boosted darunavir and dolutegravir dual therapy among a cohort of highly treatment-experienced individuals. *Antivir Ther* 2019 2019/09/21. DOI: 10.3851/IMP3330.
122. Lanzafame M, Nicole S, Gibellini D, et al. Dolutegravir monotherapy in HIV-infected naive patients with an HIV-RNA load <100 000 copies/mL: a medium-term follow-up. *J Antimicrob Chemother* 2017; 72: 2136-2138. 2017/04/08. DOI: 10.1093/jac/dkx109.
123. Rojas J, Blanco JL, Marcos MA, et al. Dolutegravir monotherapy in HIV-infected patients with sustained viral suppression. *J Antimicrob Chemother* 2016; 71: 1975-1981. 2016/03/30. DOI: 10.1093/jac/dkw078.

124. Patel DA, Snedecor SJ, Tang WY, et al. 48-week efficacy and safety of dolutegravir relative to commonly used third agents in treatment-naive HIV-1-infected patients: a systematic review and network meta-analysis. *PLoS One* 2014; 9: e105653. DOI: 10.1371/journal.pone.0105653.
125. de Boer MG and Brinkman K. Recent observations on intolerance of dolutegravir: differential causes and consequences. *AIDS* 2017; 31: 868-870. DOI: 10.1097/QAD.0000000000001395.
126. Van den Berk G OJ, Blok W, van der Meche N, Regez R, Ait Moha D, et al. Unexpectedly High Rate of Intolerance for Dolutegravir in Real-Life Setting [Internet]. CROI Conference 2016; 2016 Feb 22; Boston, Massachusetts. Available from: http://www.croiconference.org/sessions/unexpectedly-high-rate-intolerance-dolutegravir-real-life-setting.
127. Hoffmann C and Llibre JM. Neuropsychiatric Adverse Events with Dolutegravir and Other Integrase Strand Transfer Inhibitors. *AIDS Rev* 2019; 21: 4-10. DOI: 10.24875/AIDSRev.19000023.
128. Scheper H, van Holten N, Hovens J, et al. Severe depression as a neuropsychiatric side effect induced by dolutegravir. *HIV Med* 2017. DOI: 10.1111/hiv.12538.
129. Cailhol J, Rouyer C, Alloui C, et al. Dolutegravir and neuropsychiatric adverse events: a continuing debate. *AIDS* 2017; 31: 2023-2024. DOI: 10.1097/QAD.0000000000001596.
130. de Boer MG, van den Berk GE, van Holten N, et al. Intolerance of dolutegravir-containing combination antiretroviral therapy regimens in real-life clinical practice. *AIDS* 2016; 30: 2831-2834. DOI: 10.1097/QAD.0000000000001279.
131. Eisner M, Fernandez C, Michie K, et al. Response letter to SEJ Todd et al. - Early clinical experience of dolutegravir in an HIV cohort in a larger teaching hospital. *Int J STD AIDS* 2017; 28: 1051-1052. DOI: 10.1177/0956462417718759.
132. Cattaneo D, Rizzardini G and Gervasoni C. Intolerance of dolutegravir-containing combination antiretroviral therapy: not just a pharmacokinetic drug interaction. *AIDS* 2017; 31: 867-868. DOI: 10.1097/QAD.0000000000001394.
133. Bonfanti P, Madeddu G, Gulminetti R, et al. Discontinuation of treatment and adverse events in an Italian cohort of patients on dolutegravir. *AIDS* 2017; 31: 455-457. DOI: 10.1097/QAD.0000000000001351.
134. Capetti AF, Di Giambenedetto S, Latini A, et al. Morning dosing for dolutegravir-related insomnia and sleep disorders. *HIV Med* 2017. DOI: 10.1111/hiv.12540.
135. Hoffmann C, Welz T, Sabranski M, et al. Higher rates of neuropsychiatric adverse events leading to dolutegravir discontinuation in women and older patients. *HIV Med* 2017; 18: 56-63. DOI: 10.1111/hiv.12468.
136. Penafiel J, de Lazzari E, Padilla M, et al. Tolerability of integrase inhibitors in a real-life setting. *J Antimicrob Chemother* 2017; 72: 1752-1759. DOI: 10.1093/jac/dkx053.
137. Cuzin L, Pugliese P, Katlama C, et al. Integrase strand transfer inhibitors and neuropsychiatric adverse events in a large prospective cohort. *J Antimicrob Chemother* 2019; 74: 754-760. 2018/12/12. DOI: 10.1093/jac/dky497.
138. Elzi L, Erb S, Furrer H, et al. Adverse events of raltegravir and dolutegravir. *AIDS* 2017; 31: 1853-1858. DOI: 10.1097/QAD.0000000000001590.

139. Todd S, Rafferty P, Walker E, et al. Early clinical experience of dolutegravir in an HIV cohort in a larger teaching hospital. *Int J STD AIDS* 2017: 956462416688127. DOI: 10.1177/0956462416688127.
140. Wood BR. Do Integrase Inhibitors Cause Weight Gain? *Clin Infect Dis* 2019. DOI: 10.1093/cid/ciz410.
141. Menard A, Meddeb L, Tissot-Dupont H, et al. Dolutegravir and weight gain: an unexpected bothering side effect? *AIDS* 2017; 31: 1499-1500. DOI: 10.1097/QAD.0000000000001495.
142. Bourgi K, Rebeiro PF, Turner M, et al. Greater Weight Gain in Treatment Naive Persons Starting Dolutegravir-Based Antiretroviral Therapy. *Clin Infect Dis* 2019. DOI: 10.1093/cid/ciz407.
143. Kerchberger AM, Sheth AN, Angert CD, et al. Integrase strand transfer inhibitors are associated with weight gain in women. Abstract 672, Conference on retroviruses and opportunistic infections (CROI) Seattle, Washington, USA, March 4–7, 2019. 2019.
144. Hill A, Waters L and Pozniak A. Are new antiretroviral treatments increasing the risks of clinical obesity? *J Virus Erad* 2019; 5: 41-43. 2019/02/26.
145. Hill AV, W.F.; Delaporte, E. *et al*. Progressive rises in weight and clinical obesity for TAF/FTC/DTG and TDF/FTC/DTG versus TDF/FTC/EFV: ADVANCE and NAMSAL trials. 10th IAS Conference on HIV Science, Mexico City, abstract MOAX0102LB, 2019.
146. Menard A, Montagnac C, Solas C, et al. Neuropsychiatric adverse effects on dolutegravir: an emerging concern in Europe. *AIDS* 2017; 31: 1201-1203. DOI: 10.1097/QAD.0000000000001459.
147. Yagura HW, H.; Nakauchi, T. et al. Discontinuation of long-term dolutegravir treatment is associated with UGT1A1 gene polymorphisms. 10th IAS Conference on HIV Science, Mexico City, abstract MOPEB239, 2019.
148. Yagura H, Watanabe D, Kushida H, et al. Impact of UGT1A1 gene polymorphisms on plasma dolutegravir trough concentrations and neuropsychiatric adverse events in Japanese individuals infected with HIV-1. *BMC Infect Dis* 2017; 17: 622. DOI: 10.1186/s12879-017-2717-x.
149. Yagura H WD, Nakauchi T, Tomishima K, Kasai D, Nishida Y,. Effect of dolutegravir plasma concentration on central nervous system side effects | CROI Conference [Internet]. 2017 Feb 13; Seattle, Washington. Available from: http://www.croiconference.org/sessions/effect-dolutegravir-plasma-concentration-central-nervous-system-side-effects. In.
150. Parant F, Miailhes P, Brunel F, et al. Dolutegravir-Related Neurological Adverse Events: A Case Report of Successful Management with Therapeutic Drug Monitoring. *Curr Drug Saf* 2018; 13: 69-71. 2018/01/19. DOI: 10.2174/1574886313666180116124046.
151. Hoffmann C WE, Schewe K. CNS toxicity of DTG is not associated with psychiatric conditions or plasma exposure. CROI, March 4–7, 2018 | Boston, USA P424. In.
152. UNAIDS. UNAIDS Data 2017, (accessed January 2018).
153. Orrell C, Hagins DP, Belonosova E, et al. Fixed-dose combination dolutegravir, abacavir, and lamivudine versus ritonavir-boosted atazanavir plus tenofovir disoproxil fumarate and emtricitabine in previously untreated women with HIV-1 infection (ARIA): week 48 results from a randomised, open-label, non-inferiority, phase 3b study. *Lancet HIV* 2017; 4: e536-e546. DOI: 10.1016/S2352-3018(17)30095-4.

154. Kintu K, Malaba T, Nakibuka J et al. RCT of dolutegravir vs efavirenz-based therapy initiated in late pregnancy: DOLPHIN-2 [abstract 40LB]. Abstract presented at: Conference on Retroviruses and Opportunistic Infections; March 4-7, 2019; Seattle, Washington.
155. Kobbe R, Schalkwijk S, Dunay G, et al. Dolutegravir in breast milk and maternal and infant plasma during breastfeeding. *AIDS* 2016; 30: 2731-2733. DOI: 10.1097/QAD.0000000000001259.
156. Momper JD, Best BM, Wang J, et al. Elvitegravir/cobicistat pharmacokinetics in pregnant and postpartum women with HIV. *AIDS* 2018; 32: 2507-2516. 2018/08/23. DOI: 10.1097/QAD.0000000000001992.
157. Bollen P, Colbers A, Schalkwijk S, et al. A comparison of the pharmacokinetics of dolutegravir during pregnancy and post- partum [abstract O_07]. Abstract presented at: International Workshop on Clinical Pharmacology of Antiviral Therapy; June 14-16, 2017; Chicago, IL.
158. Waitt CJ, Walimbwa SI, Orrell C, et al. DolPHIN-1: Dolutegravir vs efavirenz when initiating treatment in late pregnancy—an interim analysis [abstract P807]. Abstract presented at: Conference on Retroviruses and Opportunistic Infections; March 4-7, 2018; Boston, MA.
159. Lewis JM, Railton E, Riordan A, et al. Early experience of dolutegravir pharmacokinetics in pregnancy: high maternal levels and significant foetal exposure with twice-daily dosing. *AIDS* 2016; 30: 1313-1315. DOI: 10.1097/QAD.0000000000001055.
160. Mulligan N, Best BM, Wang J, et al. Dolutegravir pharmacokinetics in pregnant and postpartum women living with HIV. *AIDS* 2018; 32: 729-737. 2018/01/26. DOI: 10.1097/QAD.0000000000001755.
161. Nguyen B, Foisy MM and Hughes CA. Pharmacokinetics and Safety of the Integrase Inhibitors Elvitegravir and Dolutegravir in Pregnant Women With HIV. *Ann Pharmacother* 2019; 53: 833-844. 2019/02/12. DOI: 10.1177/1060028019830788.
162. Zash R, Holmes L, Makhema J, et al. Surveillance for neural tube defects following antiretroviral exposure from conception, the Tsepamo study (Botswana). Program and abstracts of the 22nd International AIDS Conference; July 23-27, 2018; Amsterdam, The Netherlands. Session TUSY15.
163. Zash R, Makhema J and Shapiro RL. Neural-Tube Defects with Dolutegravir Treatment from the Time of Conception. *N Engl J Med* 2018; 379: 979-981. 2018/07/25. DOI: 10.1056/NEJMc1807653.
164. World Health Organization. Potential safety issue affecting women living with HIV using dolutegravir at the time of conception. May 18, 2018. Available at: http://www.who.int/medicines/publications/drugalerts/Statement_on_DTG_18May_2018final.pdf. Accessed August 2018.
165. European Medicines Agency. New study suggests risk of birth defects in babies born to women on HIV medicine dolutegravir. May 18, 2018. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2018/05/news_detail_002956.jsp&mid=WC0b01ac058004d5c1. Accessed July 26, 2018.
166. US Food and Drug Administration. Juluca, Tivicay, Triumeq (dolutegravir): FDA to evaluate - potential risk of neural tube birth defects. May 18, 2018. Available at: https://www.fda.gov/safety/medwatch/safetyinformation/safetyalertsforhumanmedicalproducts/ucm608168.htm. Accessed August 2018.

167. Zash RH, L.; Diseko, M. *et al*. Neural tube defects by antiretroviral and HIV exposure in the Tsepamo Study, Botswana. 10th IAS Conference on HIV Science, Mexico City, abstract MOAX0105LB, 2019.
168. Weller S, Borland J, Chen S, et al. Pharmacokinetics of dolutegravir in HIV-seronegative subjects with severe renal impairment. *Eur J Clin Pharmacol* 2014; 70: 29-35. 2013/10/08. DOI: 10.1007/s00228-013-1590-9.
169. Kreft KN, Spencer CA and Raghuram A. Safety and efficacy of dolutegravir in hemodialysis. *Int J STD AIDS* 2019; 30: 530-535. 2019/05/11. DOI: 10.1177/0956462418816785.
170. Bollen PD, Rijnders BJ, Teulen MJ, et al. Dolutegravir is not removed during hemodialysis. *AIDS* 2016; 30: 1490-1491. 2016/05/12. DOI: 10.1097/QAD.0000000000001086.
171. Molto J, Graterol F, Miranda C, et al. Removal of Dolutegravir by Hemodialysis in HIV-Infected Patients with End-Stage Renal Disease. *Antimicrob Agents Chemother* 2016; 60: 2564-2566. 2016/02/10. DOI: 10.1128/AAC.03131-15.
172. Reese MJ, Savina PM, Generaux GT, et al. In vitro investigations into the roles of drug transporters and metabolizing enzymes in the disposition and drug interactions of dolutegravir, a HIV integrase inhibitor. *Drug Metab Dispos* 2013; 41: 353-361. DOI: 10.1124/dmd.112.048918.
173. Song IH, Zong J, Borland J, et al. The Effect of Dolutegravir on the Pharmacokinetics of Metformin in Healthy Subjects. *J Acquir Immune Defic Syndr* 2016; 72: 400-407. 2016/03/15. DOI: 10.1097/QAI.0000000000000983.
174. Dooley, KE.; Purdy, E.; Sayre, P., et al. Safety, tolerability, and pharmacokinetics of the HIV integrase inhibitor dolutegravir given twice daily with rifampin: results of a Phase I study among healthy subjects. 19th Conference on Retroviruses and Opportunistic Infections; March 5–8, 2012; Seattle, WA. p. Paper #148.
175. Wang X, Cerrone M, Ferretti F, et al. Pharmacokinetics of dolutegravir 100 mg once daily with rifampicin. *Int J Antimicrob Agents* 2019 2019/04/20. DOI: 10.1016/j.ijantimicag.2019.04.009.
176. Dooley, KE.; Sayre, P.; Borland, J., et al. Pharmacokinetics, safety, and tolerability of the HIV integrase inhibitor dolutegravir co-administered with rifabutin in healthy subjects. 13th International Workshop on Clinical Pharmacology of HIV Therapy; April 16–18, 2012; Barcelona, Spain. p. Abstract P_11.
177. Song I, Borland J, Min S, et al. Effects of etravirine alone and with ritonavir-boosted protease inhibitors on the pharmacokinetics of dolutegravir. *Antimicrob Agents Chemother* 2011; 55: 3517-3521. 2011/05/11. DOI: 10.1128/AAC.00073-11.
178. Song I, Borland J, Chen S, et al. Effects of enzyme inducers efavirenz and tipranavir/ritonavir on the pharmacokinetics of the HIV integrase inhibitor dolutegravir. *Eur J Clin Pharmacol* 2014; 70: 1173-1179. 2014/08/26. DOI: 10.1007/s00228-014-1732-8.
179. Kandel CE and Walmsley SL. Dolutegravir - a review of the pharmacology, efficacy, and safety in the treatment of HIV. *Drug Des Devel Ther* 2015; 9: 3547-3555. 2015/07/18. DOI: 10.2147/DDDT.S84850.
180. Gilead Sciences. Biktarvy 50 mg/200 mg/25 mg film-coated tab- lets: EU summary of product characteristics. 2018. http://www. ema.europa.eu/. Accessed 30 Oct 2018.
181. Deeks ED. Bictegravir/Emtricitabine/Tenofovir Alafenamide: A Review in HIV-1 Infection. *Drugs* 2018; 78: 1817-1828. 2018/11/22. DOI: 10.1007/s40265-018-1010-7.

182. Molina JM, Ward D, Brar I, et al. Switching to fixed-dose bictegravir, emtricitabine, and tenofovir alafenamide from dolutegravir plus abacavir and lamivudine in virologically suppressed adults with HIV-1: 48 week results of a randomised, double-blind, multicentre, active-controlled, phase 3, non-inferiority trial. *Lancet HIV* 2018; 5: e357-e365. 2018/06/22. DOI: 10.1016/S2352-3018(18)30092-4.
183. Stellbrink HJ, Arribas JR, Stephens JL, et al. Co-formulated bictegravir, emtricitabine, and tenofovir alafenamide versus dolutegravir with emtricitabine and tenofovir alafenamide for initial treatment of HIV-1 infection: week 96 results from a randomised, double-blind, multicentre, phase 3, non-inferiority trial. *Lancet HIV* 2019; 6: e364-e372. 2019/05/10. DOI: 10.1016/S2352-3018(19)30080-3.
184. Gallant J, Lazzarin A, Mills A, et al. Bictegravir, emtricitabine, and tenofovir alafenamide versus dolutegravir, abacavir, and lamivudine for initial treatment of HIV-1 infection (GS-US-380-1489): a double-blind, multicentre, phase 3, randomised controlled non-inferiority trial. *Lancet* 2017; 390: 2063-2072. 2017/09/05. DOI: 10.1016/S0140-6736(17)32299-7.
185. Daar ES, DeJesus E, Ruane P, et al. Efficacy and safety of switching to fixed-dose bictegravir, emtricitabine, and tenofovir alafenamide from boosted protease inhibitor-based regimens in virologically suppressed adults with HIV-1: 48 week results of a randomised, open-label, multicentre, phase 3, non-inferiority trial. *Lancet HIV* 2018; 5: e347-e356. 2018/06/22. DOI: 10.1016/S2352-3018(18)30091-2.
186. Wohl DA, Yazdanpanah Y, Baumgarten A, et al. Bictegravir combined with emtricitabine and tenofovir alafenamide versus dolutegravir, abacavir, and lamivudine for initial treatment of HIV-1 infection: week 96 results from a randomised, double-blind, multicentre, phase 3, non-inferiority trial. *Lancet HIV* 2019; 6: e355-e363. 2019/05/10. DOI: 10.1016/S2352-3018(19)30077-3.
187. Kityo C, Hagins D, Koenig E, et al. Switching to bictegravir/ emtricitabine/tenofovir alafenamide in women [poster no. 500]. In: Conference on Retroviruses and Opportunistic Infections. 2018.
188. Hill L, Smith SR and Karris MY. Profile of bictegravir/emtricitabine/tenofovir alafenamide fixed dose combination and its potential in the treatment of HIV-1 infection: evidence to date. *HIV AIDS (Auckl)* 2018; 10: 203-213. 2018/11/23. DOI: 10.2147/HIV.S145529.
189. Sherman EM, Worley MV, Unger NR, et al. Cobicistat: Review of a Pharmacokinetic Enhancer for HIV Infection. *Clin Ther* 2015; 37: 1876-1893. DOI: 10.1016/j.clinthera.2015.07.022.
190. Mathias AA, German P, Murray BP, et al. Pharmacokinetics and pharmacodynamics of GS-9350: a novel pharmacokinetic enhancer without anti-HIV activity. *Clin Pharmacol Ther* 2010; 87: 322-329. 2010/01/01. DOI: 10.1038/clpt.2009.228.
191. DeJesus E, Rockstroh JK, Henry K, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate versus ritonavir-boosted atazanavir plus co-formulated emtricitabine and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3, non-inferiority trial. *Lancet* 2012; 379: 2429-2438. DOI: 10.1016/S0140-6736(12)60918-0.
192. Sax PE, DeJesus E, Mills A, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomised, double-blind, phase

3 trial, analysis of results after 48 weeks. *Lancet* 2012; 379: 2439-2448. DOI: 10.1016/S0140-6736(12)60917-9.
193. Arribas JR, Pialoux G, Gathe J, et al. Simplification to coformulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus continuation of ritonavir-boosted protease inhibitor with emtricitabine and tenofovir in adults with virologically suppressed HIV (STRATEGY-PI): 48 week results of a randomised, open-label, phase 3b, non-inferiority trial. *Lancet Infect Dis* 2014; 14: 581-589. DOI: 10.1016/S1473-3099(14)70782-0.
194. Molina JM, Lamarca A, Andrade-Villanueva J, et al. Efficacy and safety of once daily elvitegravir versus twice daily raltegravir in treatment-experienced patients with HIV-1 receiving a ritonavir-boosted protease inhibitor: randomised, double-blind, phase 3, non-inferiority study. *Lancet Infect Dis* 2012; 12: 27-35. DOI: 10.1016/S1473-3099(11)70249-3.
195. Pozniak A, Flamm J, Antinori A, et al. Switching to the single-tablet regimen of elvitegravir, cobicistat, emtricitabine, and tenofovir DF from non-nucleoside reverse transcriptase inhibitor plus coformulated emtricitabine and tenofovir DF regimens: Week 96 results of STRATEGY-NNRTI. *HIV Clin Trials* 2017; 18: 141-148. DOI: 10.1080/15284336.2017.1338844.
196. Lepist EI, Phan TK, Roy A, et al. Cobicistat boosts the intestinal absorption of transport substrates, including HIV protease inhibitors and GS-7340, in vitro. *Antimicrob Agents Chemother* 2012; 56: 5409-5413. DOI: 10.1128/AAC.01089-12.
197. Xu L, Liu H, Murray BP, et al. Cobicistat (GS-9350): A Potent and Selective Inhibitor of Human CYP3A as a Novel Pharmacoenhancer. *ACS Med Chem Lett* 2010; 1: 209-213. 2010/08/12. DOI: 10.1021/ml1000257.
198. Marzolini C, Gibbons S, Khoo S, et al. Cobicistat versus ritonavir boosting and differences in the drug-drug interaction profiles with co-medications. *J Antimicrob Chemother* 2016; 71: 1755-1758. DOI: 10.1093/jac/dkw032.
199. Deeks ED. Cobicistat: a review of its use as a pharmacokinetic enhancer of atazanavir and darunavir in patients with HIV-1 infection. *Drugs* 2014; 74: 195-206. DOI: 10.1007/s40265-013-0160-x.
200. Renjifo B, van Wyk J, Salem AH, et al. Pharmacokinetic enhancement in HIV antiretroviral therapy: a comparison of ritonavir and cobicistat. *AIDS Rev* 2015; 17: 37-46. 2015/01/15.
201. Gilead Sciences International Ltd. Tybost 150 mg film-coated tablets: EU summary of product characteristics. 2019. http:// www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_ Product_Information/human/002572/WC500153014.pdf. Accessed Sept 19.
202. Ramanathan S, Warren D, Wei L, et al. Pharmacokinetic boosting of atazanavir with the pharmacoenhancer GS-9350 versus riton- avir [poster A1-1301]. 49th Interscience Conference on Antimi- crobial Agents and Chemotherapy; 12–15 Sep 2009; San Francisco (CA).
203. Kakuda TN, Van De Casteele T, Petrovic R, et al. Bioequivalence of a darunavir/cobicistat fixed-dose combination tablet versus single agents and food effect in healthy volunteers. *Antivir Ther* 2014; 19: 597-606. 2014/06/26. DOI: 10.3851/IMP2814.
204. Kakuda TN, Opsomer M, Timmers M, et al. Pharmacokinetics of darunavir in fixed-dose combination with cobicistat compared with coadministration of darunavir and ritonavir as single agents in healthy volunteers. *J Clin Pharmacol* 2014; 54: 949-957. DOI: 10.1002/jcph.290.

205. Tashima K, Crofoot G, Tomaka FL, et al. Cobicistat-boosted darunavir in HIV-1-infected adults: week 48 results of a Phase IIIb, open-label single-arm trial. *AIDS Res Ther* 2014; 11: 39. 2014/01/01. DOI: 10.1186/1742-6405-11-39.
206. Gallant JE, Koenig E, Andrade-Villanueva J, et al. Cobicistat versus ritonavir as a pharmacoenhancer of atazanavir plus emtricitabine/tenofovir disoproxil fumarate in treatment-naive HIV type 1-infected patients: week 48 results. *J Infect Dis* 2013; 208: 32-39. 2013/03/28. DOI: 10.1093/infdis/jit122.
207. Sevinsky H, Tao X, Wang R, et al. A randomized trial in healthy subjects to assess the bioequivalence of an atazanavir/cobicistat fixed-dose combination tablet versus administration as separate agents. *Antivir Ther* 2015; 20: 493-500. 2014/11/02. DOI: 10.3851/IMP2913.
208. Evotaz: Atazanavir/cobicistat 300 mg/150 mg film-coated tablets SPC. Bristol-Myers Squibb Pharmaceutical Limited, Uxbridge, UK. February 2016.
209. Mathias A, Liu HC, Warren D, et al. Relative bioavailability and pharmacokinetics of darunavir when boosted with the pharmacoenhancer GS-9350 versus ritonavir [abstract no. 28]. 11th International Workshop on Clinical Pharmacology of HIV Therapy; 7–9 Apr 2010; Sorrento.
210. Janssen-Cilag SpA. Rezolsta 800 mg/150 mg film-coated tablets: EU summary of product characteristics. 2019. https://www.ema.europa.eu/en/documents/product-information/rezolsta-epar-product-information_en.pdf. Accessed Sept 2019. .
211. Elion R, Cohen C, Gathe J, et al. Phase 2 study of cobicistat versus ritonavir each with once-daily atazanavir and fixed-dose emtricitabine/tenofovir df in the initial treatment of HIV infection. *AIDS* 2011; 25: 1881-1886. DOI: 10.1097/QAD.0b013e32834b4d48.
212. Tolson AH and Wang H. Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. *Adv Drug Deliv Rev* 2010; 62: 1238-1249. DOI: 10.1016/j.addr.2010.08.006.
213. al. GPWXMVe. Pharmacokinetics of elvite- gravir and cobicistat in subjects with severe renal impairment [abstract no. P_38]. 13th International Workshop on Clinical Pharmacology of HIV Therapy; 16–18 April 2012; Barcelona.
214. German P, Liu HC, Szwarcberg J, et al. Effect of cobicistat on glomerular filtration rate in subjects with normal and impaired renal function. *J Acquir Immune Defic Syndr* 2012; 61: 32-40. DOI: 10.1097/QAI.0b013e3182645648.
215. Lepist EI, Zhang X, Hao J, et al. Contribution of the organic anion transporter OAT2 to the renal active tubular secretion of creatinine and mechanism for serum creatinine elevations caused by cobicistat. *Kidney Int* 2014; 86: 350-357. DOI: 10.1038/ki.2014.66.
216. Stillemans G, Belkhir L, Hesselink DA, et al. Pharmacogenetic associations with cytochrome P450 in antiretroviral therapy: what does the future hold? *Expert Opin Drug Metab Toxicol* 2018; 14: 601-611. 2018/05/19. DOI: 10.1080/17425255.2018.1478964.
217. Neary M and Owen A. Pharmacogenetic considerations for HIV treatment in different ethnicities: an update. *Expert Opin Drug Metab Toxicol* 2017; 13: 1169-1181. DOI: 10.1080/17425255.2017.1391214.
218. Borges K. International Society of Genetic Genealogy. Session 2010; 3: 20.
219. Elgar G and Vavouri T. Tuning in to the signals: noncoding sequence conservation in vertebrate genomes. *Trends Genet* 2008; 24: 344-352. 2008/06/03. DOI: 10.1016/j.tig.2008.04.005.

220. Heyn P, Kalinka AT, Tomancak P, et al. Introns and gene expression: cellular constraints, transcriptional regulation, and evolutionary consequences. *Bioessays* 2015; 37: 148-154. 2014/11/18. DOI: 10.1002/bies.201400138.
221. Slatkin M. Linkage disequilibrium--understanding the evolutionary past and mapping the medical future. *Nat Rev Genet* 2008; 9: 477-485. 2008/04/23. DOI: 10.1038/nrg2361.
222. Calcagno A, Cusato J, D'Avolio A, et al. Genetic Polymorphisms Affecting the Pharmacokinetics of Antiretroviral Drugs. *Clin Pharmacokinet* 2017; 56: 355-369. DOI: 10.1007/s40262-016-0456-6.
223. Siccardi M, Olagunju A, Simiele M, et al. Class-specific relative genetic contribution for key antiretroviral drugs. *J Antimicrob Chemother* 2015; 70: 3074-3079. DOI: 10.1093/jac/dkv207.
224. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002; 359: 727-732. DOI: 10.1016/s0140-6736(02)07873-x.
225. Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008; 358: 568-579. DOI: 10.1056/NEJMoa0706135.
226. Wells PG, Mackenzie PI, Chowdhury JR, et al. Glucuronidation and the UDP-glucuronosyltransferases in health and disease. *Drug Metab Dispos* 2004; 32: 281-290. 2004/02/24. DOI: 10.1124/dmd.32.3.281.
227. Beutler E, Gelbart T and Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci U S A* 1998; 95: 8170-8174. 1998/07/08. DOI: 10.1073/pnas.95.14.8170.
228. Horsfall LJ, Zeitlyn D, Tarekegn A, et al. Prevalence of clinically relevant UGT1A alleles and haplotypes in African populations. *Ann Hum Genet* 2011; 75: 236-246. DOI: 10.1111/j.1469-1809.2010.00638.x.
229. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* 2003; 3: 136-158. 2003/06/20. DOI: 10.1038/sj.tpj.6500171.
230. Chen S, St Jean P, Borland J, et al. Evaluation of the effect of UGT1A1 polymorphisms on dolutegravir pharmacokinetics. *Pharmacogenomics* 2014; 15: 9-16. DOI: 10.2217/pgs.13.190.
231. Tsuchiya K, Hayashida T, Hamada A, et al. Brief Report: High Peak Level of Plasma Raltegravir Concentration in Patients With ABCB1 and ABCG2 Genetic Variants. *J Acquir Immune Defic Syndr* 2016; 72: 11-14. 2016/04/21. DOI: 10.1097/QAI.0000000000000893.
232. Borghetti A, Calcagno A, Lombardi F, et al. SLC22A2 variants and dolutegravir levels correlate with psychiatric symptoms in persons with HIV. *J Antimicrob Chemother* 2019; 74: 1035-1043. 2018/12/19. DOI: 10.1093/jac/dky508.
233. Public Health England. Towards elimination of HIV transmission, AIDS and HIV-related deaths in the UK - 2017 report. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/675809Towards_elimination_ofHIV_transmission_AIDS_and_HIV_related_deaths_in_the_UK.pdf.
234. HIV Among People Aged 50 and Over. Centres for Disease Control and Prevention (CDC).

235. Calcagno A, Nozza S, Muss C, et al. Ageing with HIV: a multidisciplinary review. *Infection* 2015; 43: 509-522. DOI: 10.1007/s15010-015-0795-5.
236. Marzolini C, Back D, Weber R, et al. Ageing with HIV: medication use and risk for potential drug-drug interactions. *J Antimicrob Chemother* 2011; 66: 2107-2111. DOI: 10.1093/jac/dkr248.
237. Sundermann E, Erlandson KM, Pope CN, et al. Current Challenges and Solutions in Research and Clinical Care of Older Persons Living with HIV: Findings Presented at the 9th International Workshop on HIV and Aging. 2019. DOI: 10.1089/AID.2019.0100.
238. Vollmer S, Harttgen K, Alfven T, et al. The HIV Epidemic in Sub-Saharan Africa is Aging: Evidence from the Demographic and Health Surveys in Sub-Saharan Africa. *AIDS Behav* 2017; 21: 101-113. 2016/11/12. DOI: 10.1007/s10461-016-1591-7.
239. Klotz U. Pharmacokinetics and drug metabolism in the elderly. *Drug Metab Rev* 2009; 41: 67-76. DOI: 10.1080/03602530902722679.
240. Raffi F, Rachlis A, Stellbrink HJ, et al. Once-daily dolutegravir versus raltegravir in antiretroviral-naive adults with HIV-1 infection: 48 week results from the randomised, double-blind, non-inferiority SPRING-2 study. *Lancet* 2013; 381: 735-743. DOI: 10.1016/S0140-6736(12)61853-4.
241. Hoffmann C, Welz T, Sabranski M, et al. Reply to Letter 'Morning dosing for dolutegravir-related insomnia and sleep disorders' by Capetti et al. *HIV Med* 2017. DOI: 10.1111/hiv.12539.
242. Gamaldo CE, Gamaldo A, Creighton J, et al. Evaluating sleep and cognition in HIV. *J Acquir Immune Defic Syndr* 2013; 63: 609-616. DOI: 10.1097/QAI.0b013e31829d63ab.
243. Ram S, Seirawan H, Kumar SK, et al. Prevalence and impact of sleep disorders and sleep habits in the United States. *Sleep Breath* 2010; 14: 63-70. DOI: 10.1007/s11325-009-0281-3.
244. Low Y, Goforth HW, Omonuwa T, et al. Comparison of polysomnographic data in age-, sex- and Axis I psychiatric diagnosis matched HIV-seropositive and HIV-seronegative insomnia patients. *Clin Neurophysiol* 2012; 123: 2402-2405. DOI: 10.1016/j.clinph.2012.05.004.
245. Kinirons MT and O'Mahony MS. Drug metabolism and ageing. *Br J Clin Pharmacol* 2004; 57: 540-544. DOI: 10.1111/j.1365-2125.2004.02096.x.
246. Hippisley-Cox J, Coupland C, Vinogradova Y, et al. Predicting cardiovascular risk in England and Wales: prospective derivation and validation of QRISK2. *BMJ* 2008; 336: 1475-1482. DOI: 10.1136/bmj.39609.449676.25.
247. Wang X, Penchala SD, Amara A, et al. A Validated Method for Quantification of Dolutegravir Using Ultra Performance Liquid Chromatography Coupled With UV Detection. *Ther Drug Monit* 2016; 38: 327-331. DOI: 10.1097/FTD.0000000000000286.
248. Burger D, Teulen M, Eerland J, et al. The International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma: a global proficiency testing program. *Ther Drug Monit* 2011; 33: 239-243. DOI: 10.1097/FTD.0b013e31820fa528.
249. Buysse DJ, Reynolds CF, 3rd, Monk TH, et al. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989; 28: 193-213.
250. Bastien CH, Vallieres A and Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med* 2001; 2: 297-307.

251. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991; 14: 540-545.
252. Weaver TE, Laizner AM, Evans LK, et al. An instrument to measure functional status outcomes for disorders of excessive sleepiness. *Sleep* 1997; 20: 835-843.
253. Krupp LB, LaRocca NG, Muir-Nash J, et al. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol* 1989; 46: 1121-1123.
254. Douglass AB, Bornstein R, Nino-Murcia G, et al. The Sleep Disorders Questionnaire. I: Creation and multivariate structure of SDQ. *Sleep* 1994; 17: 160-167.
255. Cysique LA, Maruff P, Darby D, et al. The assessment of cognitive function in advanced HIV-1 infection and AIDS dementia complex using a new computerised cognitive test battery. *Arch Clin Neuropsychol* 2006; 21: 185-194. DOI: 10.1016/j.acn.2005.07.011.
256. Winston A, Puls R, Kerr SJ, et al. Differences in the direction of change of cerebral function parameters are evident over three years in HIV-infected individuals electively commencing initial cART. *PLoS One* 2015; 10: e0118608. DOI: 10.1371/journal.pone.0118608.
257. D'Souza AL. Ageing and the gut. *Postgrad Med J* 2007; 83: 44-53. DOI: 10.1136/pgmj.2006.049361.
258. Mangoni AA. The impact of advancing age on P-glycoprotein expression and activity: current knowledge and future directions. *Expert Opin Drug Metab Toxicol* 2007; 3: 315-320. DOI: 10.1517/17425255.3.3.315.
259. Riva AP, A.; Rusconi, S. . Dolutegravir and unboosted atazanavir: a dual NRTI- and booster-free antiretroviral regimen simplification in HIV-1-infected patients with viral suppression. In: *International Congress of Drug Therapy in HIV Infection 23-26 October 2016, Glasgow, UK P090.*
260. Moyle GJ and Back D. Principles and practice of HIV-protease inhibitor pharmacoenhancement. *HIV Med* 2001; 2: 105-113.
261. Sorstedt E, Carlander C, Flamholc L, et al. Effect of dolutegravir in combination with Nucleoside Reverse Transcriptase Inhibitors (NRTIs) on people living with HIV who have pre-existing NRTI mutations. *Int J Antimicrob Agents* 2018; 51: 733-738. DOI: 10.1016/j.ijantimicag.2018.01.009.
262. Brunton LM. *Chapter 1: Pharmacokinetics and Pharmacodynamics in Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th Edition*. 11th Edition ed. New York: McGRAW-HILL, 2006.
263. Cohen CJ, Colson AE, Sheble-Hall AG, et al. Pilot study of a novel short-cycle antiretroviral treatment interruption strategy: 48-week results of the five-days-on, two-days-off (FOTO) study. *HIV Clin Trials* 2007; 8: 19-23. DOI: 10.1310/hct0801-19.
264. Turkova A, Moore CL, Butler K, et al. Weekends-off efavirenz-based antiretroviral therapy in HIV-infected children, adolescents and young adults (BREATHER): Extended follow-up results of a randomised, open-label, non-inferiority trial. *PLoS One* 2018; 13: e0196239. DOI: 10.1371/journal.pone.0196239.
265. Blanco JL, Whitlock G, Milinkovic A, et al. HIV integrase inhibitors: a new era in the treatment of HIV. *Expert Opin Pharmacother* 2015; 16: 1313-1324. DOI: 10.1517/14656566.2015.1044436.

266. Tivicay (Dolutegravir 50mg). ViiV Healthcare Ltd. Stockley Park West, Uxbridge, Middlesex,, UB11 1BT, UK. Accessed on 7th June 2018. Available at: https://www.medicines.org.uk/emc/product/5248.
267. Genvoya (Elvitegravir/Cobicistat 150mg/150mg). Summary of Product Characteristics. Gilead Sciences Ltd, 280 High Holborn, London, WC1V 7EE, UK. Accessed 7th May 2018. Available at: https://www.medicines.org.uk/emc/product/5063/smpc.
268. Ramanathan S, Mathias AA, German P, et al. Clinical pharmacokinetic and pharmacodynamic profile of the HIV integrase inhibitor elvitegravir. *Clin Pharmacokinet* 2011; 50: 229-244. DOI: 10.2165/11584570-000000000-00000.
269. Boffito M, Jackson A, Amara A, et al. Pharmacokinetics of once-daily darunavir-ritonavir and atazanavir-ritonavir over 72 hours following drug cessation. *Antimicrob Agents Chemother* 2011; 55: 4218-4223. DOI: 10.1128/AAC.01747-10.
270. https://www.liverpool.ac.uk/translational-medicine/research/bioanalytical-facility/about/
271. http://kkgt.nl/?page_id=248&lang=en.
272. Else L, Watson V, Tjia J, et al. Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; 878: 1455-1465. DOI: 10.1016/j.jchromb.2010.03.036.
273. Penchala SD, Fawcett S, Else L, et al. The development and application of a novel LC-MS/MS method for the measurement of Dolutegravir, Elvitegravir and Cobicistat in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2016; 1027: 174-180. DOI: 10.1016/j.jchromb.2016.05.040.
274. PREZISTA™ (Tibotec, Inc.) (Darunavir). Full prescribing information. Food and drug administration. 2008. [cited 2018 Apr 31]. Available from: http://accessdata.fda.gov/drugsatfda_docs/label/2008/021976s003s004lbl.pdf.
275. Bertz RJ, Persson A, Chung E, et al. Pharmacokinetics and pharmacodynamics of atazanavir-containing antiretroviral regimens, with or without ritonavir, in patients who are HIV-positive and treatment-naive. *Pharmacotherapy* 2013; 33: 284-294. DOI: 10.1002/phar.1205.
276. La Porte C. Updated guideline to perform therapeutic drug monitoring for antiretroviral agents. Rev Antiviral Ther 2006; **3:** 4–14.
277. Evotaz (Atazanavir/cobicistat 300 mg/150 mg), film-coated tablets. February 2016; Bristol-Myers Squibb Pharmaceutical Limited, Uxbridge, UK. Accessed 20 May 2018. Available at: https://www.medicines.org.uk/emc/product/7006.
278. Brainard DM, Wenning LA, Stone JA, et al. Clinical pharmacology profile of raltegravir, an HIV-1 integrase strand transfer inhibitor. *J Clin Pharmacol* 2011; 51: 1376-1402. DOI: 10.1177/0091270010387428.
279. Moyle G, Boffito M, Fletcher C, et al. Steady-state pharmacokinetics of abacavir in plasma and intracellular carbovir triphosphate following administration of abacavir at 600 milligrams once daily and 300 milligrams twice daily in human immunodeficiency virus-infected subjects. *Antimicrob Agents Chemother* 2009; 53: 1532-1538. DOI: 10.1128/AAC.01000-08.
280. Hawkins T VW, StClaire R, Hey A, Guyer B, Kearney BP. Intracellular pharmacokinetics of tenofovir-DP and carbovir-TP in patients receiving triple nucleoside regimens [abstract 2.4]. *5th International Workshop on Clinical Pharmacology of HIV Therapy*. Rome, Italy. 1-3 April 2004.

281. Robbins BL, Srinivas RV, Kim C, et al. Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(isopropyloxymethylcarbonyl)PMPA. *Antimicrob Agents Chemother* 1998; 42: 612-617.
282. Stevens RC, Blum MR, Rousseau FS, et al. Intracellular pharmacology of emtricitabine and tenofovir. *Clin Infect Dis* 2004; 39: 877-878; author reply 878-879. DOI: 10.1086/423810.
283. Wang LH BJ, Feng JY, Quinn J, Rousseau F. *Pharmacokinetic and pharmacodynamic characteristics of emtricitabine support its once daily dosing [abstract 4546] XIV International AIDS Conference Barcelona, Spain; July 7–12, 2002*.
284. Ziagen (abacavir). Summary of Product Characteristics. London: ViiV. Healthcare Ltd. Stockley Park West, Uxbridge, Middlesex, UB11 1BT, UK. 21 July 2016. Accessed on 2 May 2018. Available at: https://www.medicines.org.uk/emc/product/5518/smpc.
285. Mathias A LH, Warren D, Sekar V, Kearney BP. Relative bioavailability and pharmacokinetics of darunavir when boosted with the pharmacoenhancer GS-9350 versus RTV [abstract #28]. 1*1th International Workshop on Clinical Pharmacology of HIV Therapy*. Sorrento, Italy. April 7–9 2010.
286. De Meyer S, Azijn H, Surleraux D, et al. TMC114, a novel human immunodeficiency virus type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates. *Antimicrob Agents Chemother* 2005; 49: 2314-2321. 2005/05/27. DOI: 10.1128/AAC.49.6.2314-2321.2005.
287. Sekar V, et al. Absence of TMC114 exposure-efficacy and exposuresafety relationships in POWER 3. abstr. TUPE0078. XVI Int. AIDS Conf., Toronto, Canada, 13 to 18 August 2006.
288. Dickinson L, Khoo S and Back D. Differences in the pharmacokinetics of protease inhibitors between healthy volunteers and HIV-infected persons. *Curr Opin HIV AIDS* 2008; 3: 296-305. DOI: 10.1097/COH.0b013e3282f82bf1.
289. Coleman CI, Roberts MS, Sobieraj DM, et al. Effect of dosing frequency on chronic cardiovascular disease medication adherence. *Curr Med Res Opin* 2012; 28: 669-680. DOI: 10.1185/03007995.2012.677419.
290. Livio F and Marzolini C. Prescribing issues in older adults living with HIV: thinking beyond drug-drug interactions with antiretroviral drugs. *Ther Adv Drug Saf* 2019; 10: 2042098619880122. 2019/10/18. DOI: 10.1177/2042098619880122.
291. Arasteh K, Yeni P, Pozniak A, et al. Efficacy and safety of darunavir/ritonavir in treatment-experienced HIV type-1 patients in the POWER 1, 2 and 3 trials at week 96. *Antivir Ther* 2009; 14: 859-864. DOI: 10.3851/IMP1301.
292. Elliot E, Amara A, Jackson A, et al. Dolutegravir and elvitegravir plasma concentrations following cessation of drug intake. *J Antimicrob Chemother* 2016; 71: 1031-1036. DOI: 10.1093/jac/dkv425.
293. Tashima KT, Mollan KR, Na L, et al. Regimen selection in the OPTIONS trial of HIV salvage therapy: drug resistance, prior therapy, and race-ethnicity determine the degree of regimen complexity. *HIV Clin Trials* 2015; 16: 147-156. DOI: 10.1179/1945577115Y.0000000001.
294. Pulido F, Ribera E, Lagarde M, et al. Dual Therapy With Darunavir and Ritonavir Plus Lamivudine vs Triple Therapy With Darunavir and Ritonavir Plus Tenofovir Disoproxil Fumarate and Emtricitabine or Abacavir and Lamivudine for

Maintenance of Human Immunodeficiency Virus Type 1 Viral Suppression: Randomized, Open-Label, Noninferiority DUAL-GESIDA 8014-RIS-EST45 Trial. *Clin Infect Dis* 2017; 65: 2112-2118. DOI: 10.1093/cid/cix734.
295. Sued O FM, Gun A, Belloso W, Cecchini D, Lopardo G. Dual therapy with darunavir/ritonavir plus lamivudine for HIV-1 treatment initiation: week 24 results of the randomized ANDES study. 9th International AIDS Society (IAS) Conference on HIV Science (IAS 2017). Paris, 23–26th July 2017. Oral Abstract MOAB0106LB.
296. Taiwo BO ZL, Nyaku AN, Stefanescu A, Sax PE, Haas D et al. ACTG A5353: a pilot study of dolutegravir (DTG) + lamivudine (3TC) for initial treatment of HIV-1-infected participants with HIV-1 RNA < 500,000 copies/mL. 9th International AIDS Society (IAS) Conference on HIV Science (IAS 2017). Paris, 23–26th July 2017. Oral Abstract MOAB0107LB.
297. Revuelta-Herrero JL, Chamorro-de-Vega E, Rodriguez-Gonzalez CG, et al. Effectiveness, Safety, and Costs of a Treatment Switch to Dolutegravir Plus Rilpivirine Dual Therapy in Treatment-Experienced HIV Patients. *Ann Pharmacother* 2018; 52: 11-18. 2017/08/25. DOI: 10.1177/1060028017728294.
298. Lambert-Niclot S, George EC, Pozniak A, et al. Antiretroviral resistance at virological failure in the NEAT 001/ANRS 143 trial: raltegravir plus darunavir/ritonavir or tenofovir/emtricitabine plus darunavir/ritonavir as first-line ART. *J Antimicrob Chemother* 2016; 71: 1056-1062. 2015/12/26. DOI: 10.1093/jac/dkv427.
299. Raffi F, Babiker AG, Richert L, et al. Ritonavir-boosted darunavir combined with raltegravir or tenofovir-emtricitabine in antiretroviral-naive adults infected with HIV-1: 96 week results from the NEAT001/ANRS143 randomised non-inferiority trial. *Lancet* 2014; 384: 1942-1951. DOI: 10.1016/S0140-6736(14)61170-3.
300. Nishijima T, Gatanaga H, Shimbo T, et al. Switching tenofovir/emtricitabine plus lopinavir/r to raltegravir plus Darunavir/r in patients with suppressed viral load did not result in improvement of renal function but could sustain viral suppression: a randomized multicenter trial. *PLoS One* 2013; 8: e73639. 2013/08/21. DOI: 10.1371/journal.pone.0073639.
301. Dierynck I, De Wit M, Gustin E, et al. Binding kinetics of darunavir to human immunodeficiency virus type 1 protease explain the potent antiviral activity and high genetic barrier. *J Virol* 2007; 81: 13845-13851. DOI: 10.1128/JVI.01184-07.
302. Capetti AF, Sterrantino G, Cossu MV, et al. Salvage therapy or simplification of salvage regimens with dolutegravir plus ritonavir-boosted darunavir dual therapy in highly cART-experienced subjects: an Italian cohort. *Antivir Ther* 2017; 22: 257-262. DOI: 10.3851/IMP3095.
303. Anstett K, Brenner B, Mesplede T, et al. HIV drug resistance against strand transfer integrase inhibitors. *Retrovirology* 2017; 14: 36. 2017/06/07. DOI: 10.1186/s12977-017-0360-7.
304. Spinner CD, Kummerle T, Krznaric I, et al. Pharmacokinetics of once-daily dolutegravir and ritonavir-boosted darunavir in HIV patients: the DUALIS study. *J Antimicrob Chemother* 2017; 72: 2679-2681. DOI: 10.1093/jac/dkx105.
305. Gervasoni C, Riva A, Cozzi V, et al. Effects of ritonavir and cobicistat on dolutegravir exposure: when the booster can make the difference. *J Antimicrob Chemother* 2017; 72: 1842-1844. DOI: 10.1093/jac/dkx055.
306. Song I, Min SS, Borland J, et al. The effect of lopinavir/ritonavir and darunavir/ritonavir on the HIV integrase inhibitor S/GSK1349572 in healthy

participants. *J Clin Pharmacol* 2011; 51: 237-242. DOI: 10.1177/0091270010371113.
307. Gutierrez F, Fulladosa X, Barril G, et al. Renal tubular transporter-mediated interactions of HIV drugs: implications for patient management. *AIDS Rev* 2014; 16: 199-212.
308. Milburn J, Jones R and Levy JB. Renal effects of novel antiretroviral drugs. *Nephrol Dial Transplant* 2017; 32: 434-439. DOI: 10.1093/ndt/gfw064.
309. Moore CG, Carter RE, Nietert PJ, et al. Recommendations for planning pilot studies in clinical and translational research. *Clin Transl Sci* 2011; 4: 332-337. 2011/10/28. DOI: 10.1111/j.1752-8062.2011.00347.x.
310. Algeelani S, Alam N, Hossain MA, et al. In vitro inhibition of human UGT isoforms by ritonavir and cobicistat. *Xenobiotica* 2018; 48: 764-769. 2017/09/12. DOI: 10.1080/00498254.2017.1370655.
311. UNAIDS. Data 2017. http://www.unaids.org/sites/default/files/media_asset/20170720_Data_book_2017_en.pdf.
312. Haddad LB, Monsour M, Tepper NK, et al. Trends in contraceptive use according to HIV status among privately insured women in the United States. *Am J Obstet Gynecol* 2017; 217: 676 e671-676 e611. DOI: 10.1016/j.ajog.2017.08.006.
313. Kaida A, Patterson S, Carter A, et al. Contraceptive Choice and Use of Dual Protection Among Women Living with HIV in Canada: Priorities for Integrated Care. *Perspect Sex Reprod Health* 2017; 49: 223-236. DOI: 10.1363/psrh.12046.
314. Kennedy VL, Serghides L, Raboud JM, et al. The importance of motherhood in HIV-positive women of reproductive age in Ontario, Canada. *AIDS Care* 2014; 26: 777-784. DOI: 10.1080/09540121.2013.855295.
315. Nostlinger C, Desjardins F, Dec J, et al. Child desire in women and men living with HIV attending HIV outpatient clinics: evidence from a European multicentre study. *Eur J Contracept Reprod Health Care* 2013; 18: 251-263. DOI: 10.3109/13625187.2013.801072.
316. Aizire J, Yende N, Nematadzira T, et al. High frequency of unintended pregnancy and predictors of contraceptive choice among HIV-infected African women on life-long antiretroviral therapy (ART). The US-PEPFAR PROMOTE Cohort Study. 22nd International AIDS Conference (AIDS 2018), Amsterdam, abstract THAB0301, July 2018. In.
317. Daniels K, Daugherty J, Jones J, et al. Current Contraceptive Use and Variation by Selected Characteristics Among Women Aged 15-44: United States, 2011-2013. *Natl Health Stat Report* 2015: 1-14.
318. Statistics on Sexual and Reproductive Health Services; England 2015/16 .
319. Thurman AR, Anderson S and Doncel GF. Effects of hormonal contraception on antiretroviral drug metabolism, pharmacokinetics and pharmacodynamics. *Am J Reprod Immunol* 2014; 71: 523-530. DOI: 10.1111/aji.12210.
320. McCann MF and Potter LS. Progestin-only oral contraception: a comprehensive review. *Contraception* 1994; 50: S1-195.
321. Phillips S, Steyn P and Temmerman M. Contraceptive options for women living with HIV. *Best Pract Res Clin Obstet Gynaecol* 2014; 28: 881-890. DOI: 10.1016/j.bpobgyn.2014.04.013.
322. Pyra M, Heffron R, Mugo NR, et al. Effectiveness of hormonal contraception in HIV-infected women using antiretroviral therapy. *AIDS* 2015; 29: 2353-2359. DOI: 10.1097/QAD.0000000000000827.

323. Dreher HM. The effect of caffeine reduction on sleep quality and well-being in persons with HIV. *J Psychosom Res* 2003; 54: 191-198.
324. Patterson S, Carter A, Nicholson V, et al. Condomless Sex Among Virally Suppressed Women With HIV With Regular HIV-Serodiscordant Sexual Partners in the Era of Treatment as Prevention. *J Acquir Immune Defic Syndr* 2017; 76: 372-381. DOI: 10.1097/QAI.0000000000001528.
325. Radix A, Sevelius J and Deutsch MB. Transgender women, hormonal therapy and HIV treatment: a comprehensive review of the literature and recommendations for best practices. *J Int AIDS Soc* 2016; 19: 20810. DOI: 10.7448/IAS.19.3.20810.
326. Baral SD, Poteat T, Stromdahl S, et al. Worldwide burden of HIV in transgender women: a systematic review and meta-analysis. *Lancet Infect Dis* 2013; 13: 214-222. DOI: 10.1016/S1473-3099(12)70315-8.
327. Kellogg TA, Clements-Nolle K, Dilley J, et al. Incidence of human immunodeficiency virus among male-to-female transgendered persons in San Francisco. *J Acquir Immune Defic Syndr* 2001; 28: 380-384.
328. Baguso GN, Gay CL and Lee KA. Medication adherence among transgender women living with HIV. *AIDS Care* 2016; 28: 976-981. DOI: 10.1080/09540121.2016.1146401.
329. Santos GM, Wilson EC, Rapues J, et al. HIV treatment cascade among transgender women in a San Francisco respondent driven sampling study. *Sex Transm Infect* 2014; 90: 430-433. DOI: 10.1136/sextrans-2013-051342.
330. Sevelius JM, Patouhas E, Keatley JG, et al. Barriers and facilitators to engagement and retention in care among transgender women living with human immunodeficiency virus. *Ann Behav Med* 2014; 47: 5-16. DOI: 10.1007/s12160-013-9565-8.
331. Gradinger F, Cieza A, Stucki A, et al. Part 1. International Classification of Functioning, Disability and Health (ICF) Core Sets for persons with sleep disorders: results of the consensus process integrating evidence from preparatory studies. *Sleep Med* 2011; 12: 92-96. DOI: 10.1016/j.sleep.2010.08.009.
332. Winter S and Doussantousse S. Transpeople, hormones, and health risks in Southeast Asia: a lao study. Int J Sex Health. 2009;21(1):3548.
333. Battegay M, Lundgren JD and Ryom L. European AIDS Clinical Society. Clinical management and treatment of HIV-infected patients in Europe. V 9.0. October 2017. Available from: http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html. Accessed 4 July 2018.
334. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015 (2016 interim update). http://www.bhiva.org/documents/Guidelines/Treatment/2016/treatment-guidelines-2016-interim-update.pdf. BHIVA. Accessed 19 December 2017.
335. Elliot ER, Amara A, Pagani N, et al. Once-daily atazanavir/cobicistat and darunavir/cobicistat exposure over 72 h post-dose in plasma, urine and saliva: contribution to drug pharmacokinetic knowledge. *J Antimicrob Chemother* 2017; 72: 2143. DOI: 10.1093/jac/dkx145.
336. Tittle V, Bull L, Boffito M, et al. Pharmacokinetic and pharmacodynamic drug interactions between antiretrovirals and oral contraceptives. *Clin Pharmacokinet* 2015; 54: 23-34. DOI: 10.1007/s40262-014-0204-8.
337. Atrio J, Stanczyk FZ, Neely M, et al. Effect of protease inhibitors on steady-state pharmacokinetics of oral norethindrone contraception in HIV-infected women. *J Acquir Immune Defic Syndr* 2014; 65: 72-77. DOI: 10.1097/QAI.0b013e3182a9b3f1.

338. Zhang J, Chung E, Yones C, et al. The effect of atazanavir/ritonavir on the pharmacokinetics of an oral contraceptive containing ethinyl estradiol and norgestimate in healthy women. *Antivir Ther* 2011; 16: 157-164. DOI: 10.3851/IMP1724.
339. Tackett D CM, Agarwala S, et al. Atazanavir: a summary of two pharmacokinetic drug interaction studies in healthy subjects. In: *10th Conference on Retrovirus and Opportunistic Infections* Boston, 10–14 Feb 2003.
340. Majeed SR, West SK, Shuping J, et al. Confirmation of the Drug-drug Interaction Potential Between Cobicistat-boosted Antiretroviral Regimen and Hormonal Contraceptives. In: *IWCPAT* Chicago, 2017.
341. Blode H, Wuttke W, Loock W, et al. A 1-year pharmacokinetic investigation of a novel oral contraceptive containing drospirenone in healthy female volunteers. *Eur J Contracept Reprod Health Care* 2000; 5: 256-264.
342. Cartwright RD. Alcohol and NREM parasomnias: evidence versus opinions in the international classification of sleep disorders, 3rd edition. *J Clin Sleep Med* 2014; 10: 1039-1040. DOI: 10.5664/jcsm.4050.
343. Cirrincione LR, Penchala SD, Scarsi KK, et al. Development, validation and utilization of a highly sensitive LC-MS/MS method for quantification of levonorgestrel released from a subdermal implant in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2018; 1084: 106-112. DOI: 10.1016/j.jchromb.2018.03.023.
344. Squires K, Kityo C, Hodder S, et al. Integrase inhibitor versus protease inhibitor based regimen for HIV-1 infected women (WAVES): a randomised, controlled, double-blind, phase 3 study. *Lancet HIV* 2016; 3: e410-e420. DOI: 10.1016/S2352-3018(16)30016-9.
345. Watkins PB. Drug metabolism by cytochromes P450 in the liver and small bowel. *Gastroenterol Clin North Am* 1992; 21: 511-526.
346. Spatzenegger M and Jaeger W. Clinical importance of hepatic cytochrome P450 in drug metabolism. *Drug Metab Rev* 1995; 27: 397-417. DOI: 10.3109/03602539508998329.
347. Durnas C, Loi CM and Cusack BJ. Hepatic drug metabolism and aging. *Clin Pharmacokinet* 1990; 19: 359-389. DOI: 10.2165/00003088-199019050-00002.
348. Rivera R, Yacobson I and Grimes D. The mechanism of action of hormonal contraceptives and intrauterine contraceptive devices. *Am J Obstet Gynecol* 1999; 181: 1263-1269.
349. Sondheimer SJ. Oral contraceptives: mechanism of action, dosing, safety, and efficacy. *Cutis* 2008; 81: 19-22.
350. Olsson B and Landgren BM. The effect of tolterodine on the pharmacokinetics and pharmacodynamics of a combination oral contraceptive containing ethinyl estradiol and levonorgestrel. *Clin Ther* 2001; 23: 1876-1888.
351. Teofilo E, Rocha-Pereira N, Kuhlmann B, et al. Long-Term Efficacy, Tolerability, and Renal Safety of Atazanavir/Ritonavir-based Antiretroviral Therapy in a Cohort of Treatment-Naive Patients with HIV-1 Infection: the REMAIN Study. *HIV Clin Trials* 2016; 17: 17-28. DOI: 10.1080/15284336.2015.1112494.
352. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at http://www.aidsinfo.nih.gov/ContentFiles/ AdultandAdolescentGL.pdf. Accessed May 2018.

353. Sax PE, Erlandson KM, Lake JE, et al. Weight Gain Following Initiation of Antiretroviral Therapy: Risk Factors in Randomized Comparative Clinical Trials. *Clin Infect Dis* 2019 2019/10/14. DOI: 10.1093/cid/ciz999.
354. Elliot ER, Wang X, Singh S, et al. Increased dolutegravir peak concentrations in people living with HIV aged 60 and over and analysis of sleep quality and cognition. *Clin Infect Dis* 2018. DOI: 10.1093/cid/ciy426.
355. Capetti AF, Astuti N, Cattaneo D, et al. Pharmacokinetic drug evaluation of dolutegravir plus rilpivirine for the treatment of HIV. *Expert Opin Drug Metab Toxicol* 2017; 13: 1183-1192. 2017/09/01. DOI: 10.1080/17425255.2017.1361929.
356. Cattaneo D, Capetti A and Rizzardini G. Drug-drug interactions of a two-drug regimen of dolutegravir and lamivudine for HIV treatment. *Expert Opin Drug Metab Toxicol* 2019; 15: 245-252. 2019/02/02. DOI: 10.1080/17425255.2019.1577821.
357. Tsuchiya K, Hayashida T, Hamada A, et al. High plasma concentrations of dolutegravir in patients with ABCG2 genetic variants. *Pharmacogenet Genomics* 2017; 27: 416-419. DOI: 10.1097/FPC.0000000000000308.
358. Wang D, Guo Y, Wrighton SA, et al. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenomics J* 2011; 11: 274-286. DOI: 10.1038/tpj.2010.28.
359. Olagunju A, Schipani A, Siccardi M, et al. CYP3A4*22 (c.522-191 C>T; rs35599367) is associated with lopinavir pharmacokinetics in HIV-positive adults. *Pharmacogenet Genomics* 2014; 24: 459-463. DOI: 10.1097/FPC.0000000000000073.
360. Russo G, Paganotti GM, Soeria-Atmadja S, et al. Pharmacogenetics of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in resource-limited settings: Influence on antiretroviral therapy response and concomitant anti-tubercular, antimalarial and contraceptive treatments. *Infect Genet Evol* 2016; 37: 192-207. 2015/11/26. DOI: 10.1016/j.meegid.2015.11.014.
361. Savic RM, Barrail-Tran A, Duval X, et al. Effect of adherence as measured by MEMS, ritonavir boosting, and CYP3A5 genotype on atazanavir pharmacokinetics in treatment-naive HIV-infected patients. *Clin Pharmacol Ther* 2012; 92: 575-583. 2012/10/04. DOI: 10.1038/clpt.2012.137.
362. Sugatani J, Uchida T, Kurosawa M, et al. Regulation of pregnane X receptor (PXR) function and UGT1A1 gene expression by posttranslational modification of PXR protein. *Drug Metab Dispos* 2012; 40: 2031-2040. DOI: 10.1124/dmd.112.046748.
363. Siccardi M, D'Avolio A, Baietto L, et al. Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C-->T) with reduced concentrations of unboosted atazanavir. *Clin Infect Dis* 2008; 47: 1222-1225. DOI: 10.1086/592304.
364. Schipani A, Siccardi M, D'Avolio A, et al. Population pharmacokinetic modeling of the association between 63396C->T pregnane X receptor polymorphism and unboosted atazanavir clearance. *Antimicrob Agents Chemother* 2010; 54: 5242-5250. DOI: 10.1128/AAC.00781-10.
365. Rodriguez S, Gaunt TR and Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009; 169: 505-514. DOI: 10.1093/aje/kwn359.
366. Zhao F, Song M, Wang Y, et al. Genetic model. *J Cell Mol Med* 2016; 20: 765. 2016/01/15. DOI: 10.1111/jcmm.12751.

367. Trontelj J, Marc J, Zavratnik A, et al. Effects of UGT1A1*28 polymorphism on raloxifene pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol* 2009; 67: 437-444. 2009/04/18. DOI: 10.1111/j.1365-2125.2009.03363.x.
368. Hu ZY, Yu Q, Pei Q, et al. Dose-dependent association between UGT1A1*28 genotype and irinotecan-induced neutropenia: low doses also increase risk. *Clin Cancer Res* 2010; 16: 3832-3842. 2010/06/22. DOI: 10.1158/1078-0432.CCR-10-1122.
369. Wenning LA, Petry AS, Kost JT, et al. Pharmacokinetics of raltegravir in individuals with UGT1A1 polymorphisms. *Clin Pharmacol Ther* 2009; 85: 623-627. DOI: 10.1038/clpt.2009.12.
370. Lankisch TO, Behrens G, Ehmer U, et al. Gilbert's syndrome and hyperbilirubinemia in protease inhibitor therapy--an extended haplotype of genetic variants increases risk in indinavir treatment. *J Hepatol* 2009; 50: 1010-1018. DOI: 10.1016/j.jhep.2008.12.030.
371. Khoury MJ. Genetics and genomics in practice: the continuum from genetic disease to genetic information in health and disease. *Genet Med* 2003; 5: 261-268. DOI: 10.1097/01.GIM.0000076977.90682.A5.
372. Gharani N, Keller MA, Stack CB, et al. The Coriell personalized medicine collaborative pharmacogenomics appraisal, evidence scoring and interpretation system. *Genome Med* 2013; 5: 93. DOI: 10.1186/gm499.
373. Tonk ECM, Gurwitz D, Maitland-van der Zee AH, et al. Assessment of pharmacogenetic tests: presenting measures of clinical validity and potential population impact in association studies. *Pharmacogenomics J* 2017; 17: 386-392. DOI: 10.1038/tpj.2016.34.
374. Kondo C, Suzuki H, Itoda M, et al. Functional analysis of SNPs variants of BCRP/ABCG2. *Pharm Res* 2004; 21: 1895-1903.
375. Birmingham BK, Bujac SR, Elsby R, et al. Impact of ABCG2 and SLCO1B1 polymorphisms on pharmacokinetics of rosuvastatin, atorvastatin and simvastatin acid in Caucasian and Asian subjects: a class effect? *Eur J Clin Pharmacol* 2015; 71: 341-355. DOI: 10.1007/s00228-014-1801-z.
376. Mizuno T, Fukudo M, Terada T, et al. Impact of genetic variation in breast cancer resistance protein (BCRP/ABCG2) on sunitinib pharmacokinetics. *Drug Metab Pharmacokinet* 2012; 27: 631-639.
377. Modongo C, Wang Q, Dima M, et al. Clinical and Virological Outcomes of TB/HIV Coinfected Patients Treated With Dolutegravir-Based HIV Antiretroviral Regimens: Programmatic Experience From Botswana. *J Acquir Immune Defic Syndr* 2019; 82: 111-115. 2019/07/25. DOI: 10.1097/QAI.0000000000002126.
378. Singh K, Sarafianos SG and Sonnerborg A. Long-Acting Anti-HIV Drugs Targeting HIV-1 Reverse Transcriptase and Integrase. *Pharmaceuticals (Basel)* 2019; 12 2019/04/24. DOI: 10.3390/ph12020062.
379. Salter ML, Lau B, Go VF, et al. HIV infection, immune suppression, and uncontrolled viremia are associated with increased multimorbidity among aging injection drug users. *Clin Infect Dis* 2011; 53: 1256-1264. 2011/10/07. DOI: 10.1093/cid/cir673.
380. Di Angelantonio E, Chowdhury R, Sarwar N, et al. Chronic kidney disease and risk of major cardiovascular disease and non-vascular mortality: prospective population based cohort study. *BMJ* 2010; 341: c4986. 2010/10/05. DOI: 10.1136/bmj.c4986.

381. Ryom L, Lundgren JD, El-Sadr W, et al. Cardiovascular disease and use of contemporary protease inhibitors: the D:A:D international prospective multicohort study. *Lancet HIV* 2018; 5: e291-e300. DOI: 10.1016/S2352-3018(18)30043-2.
382. Ryom L, Dilling Lundgren J, Reiss P, et al. Use of Contemporary Protease Inhibitors and Risk of Incident Chronic Kidney Disease in Persons With Human Immunodeficiency Virus: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study. *J Infect Dis* 2019; 220: 1629-1634. 2019/09/11. DOI: 10.1093/infdis/jiz369.
383. Group DADS, Sabin CA, Worm SW, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet* 2008; 371: 1417-1426. 2008/04/05. DOI: 10.1016/S0140-6736(08)60423-7.
384. Boyd MA, Mocroft A, Ryom L, et al. Cardiovascular disease (CVD) and chronic kidney disease (CKD) event rates in HIV-positive persons at high predicted CVD and CKD risk: A prospective analysis of the D:A:D observational study. *PLoS Med* 2017; 14: e1002424. 2017/11/08. DOI: 10.1371/journal.pmed.1002424.
385. Coetzee L, Bogler L, De Neve JW, et al. HIV, antiretroviral therapy and non-communicable diseases in sub-Saharan Africa: empirical evidence from 44 countries over the period 2000 to 2016. *J Int AIDS Soc* 2019; 22: e25364. 2019/07/30. DOI: 10.1002/jia2.25364.
386. Demessine L, Peyro-Saint-Paul L, Gardner EM, et al. Risk and Cost Associated With Drug-Drug Interactions Among Aging HIV Patients Receiving Combined Antiretroviral Therapy in France. *Open Forum Infect Dis* 2019; 6: ofz051. 2019/04/06. DOI: 10.1093/ofid/ofz051.
387. Mathis S, Khanlari B, Pulido F, et al. Effectiveness of protease inhibitor monotherapy versus combination antiretroviral maintenance therapy: a meta-analysis. *PLoS One* 2011; 6: e22003. 2011/08/04. DOI: 10.1371/journal.pone.0022003.
388. Clement ME, Kofron R and Landovitz RJ. Long-acting injectable cabotegravir for the prevention of HIV infection. *Curr Opin HIV AIDS* 2019 2019/10/24. DOI: 10.1097/COH.0000000000000597.
389. Hill A, Venter W.F, Delaporte E, *et al.* Progressive rises in weight and clinical obesity for TAF/FTC/DTG and TDF/FTC/DTG versus TDF/FTC/EFV: ADVANCE and NAMSAL trials. IAS 2019, Mexico City, Mexico. Oral session - MOAX0102LB.
390. European Medicines Agency. Assessment report. Dolutegravir (Tivicay) Available at: www.ema.europa.eu/documents/assessment-report/tivicay-epar-public-assessment- report_en.pdf (accessed January 2019).
391. Acosta EP, Limoli KL, Trinh L, et al. Novel method to assess antiretroviral target trough concentrations using in vitro susceptibility data. *Antimicrob Agents Chemother* 2012; 56: 5938-5945. 2012/09/12. DOI: 10.1128/AAC.00691-12.
392. van Lunzen J, Maggiolo F, Arribas JR, et al. Once daily dolutegravir (S/GSK1349572) in combination therapy in antiretroviral-naive adults with HIV: planned interim 48 week results from SPRING-1, a dose-ranging, randomised, phase 2b trial. *Lancet Infect Dis* 2012; 12: 111-118. 2011/10/25. DOI: 10.1016/S1473-3099(11)70290-0.
393. Punyawudho B, Thammajaruk N, Ruxrungtham K, et al. Population pharmacokinetics and dose optimisation of ritonavir-boosted atazanavir in Thai HIV-infected patients. *Int J Antimicrob Agents* 2017; 49: 327-332. 2017/01/23. DOI: 10.1016/j.ijantimicag.2016.11.019.

394. Rougemont M, Nchotu Ngang P, Stoll B, et al. Safety of zidovudine dose reduction in treatment-naive HIV infected patients. A randomized controlled study (MiniZID). *HIV Med* 2016; 17: 206-215. 2015/09/12. DOI: 10.1111/hiv.12303.
395. Dickinson L, Amin J, Else L, et al. Pharmacokinetic and Pharmacodynamic Comparison of Once-Daily Efavirenz (400 mg vs. 600 mg) in Treatment-Naive HIV-Infected Patients: Results of the ENCORE1 Study. *Clin Pharmacol Ther* 2015; 98: 406-416. 2015/06/06. DOI: 10.1002/cpt.156.
396. Dickinson L, Amin J, Else L, et al. Comprehensive Pharmacokinetic, Pharmacodynamic and Pharmacogenetic Evaluation of Once-Daily Efavirenz 400 and 600 mg in Treatment-Naive HIV-Infected Patients at 96 Weeks: Results of the ENCORE1 Study. *Clin Pharmacokinet* 2016; 55: 861-873. 2015/12/31. DOI: 10.1007/s40262-015-0360-5.
397. Lamorde M, Wang X, Neary M, et al. Pharmacokinetics, Pharmacodynamics, and Pharmacogenetics of Efavirenz 400 mg Once Daily During Pregnancy and Post-Partum. *Clin Infect Dis* 2018; 67: 785-790. 2018/08/21. DOI: 10.1093/cid/ciy161.
398. Kaboggoza JP, Wang X, Neary M, et al. A Lower Dose of Efavirenz Can Be Coadministered With Rifampicin and Isoniazid in Tuberculosis Patients. *Open Forum Infect Dis* 2019; 6: ofz035. 2019/03/01. DOI: 10.1093/ofid/ofz035.
399. Cerrone M, Wang X, Neary M, et al. Pharmacokinetics of Efavirenz 400 mg Once Daily Coadministered With Isoniazid and Rifampicin in Human Immunodeficiency Virus-Infected Individuals. *Clin Infect Dis* 2019; 68: 446-452. 2018/08/08. DOI: 10.1093/cid/ciy491.